Diagnostic Protocols in Endocrinology

Sanjay Bhadada
Liza Das
Rimesh Pal
Editors

Springer

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A L G r a w a n y

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Foreword

The science of endocrinology is unique with its innate ability to measure hormones, indeed a major revolutionary discovery. This has had tremendous impact on the diagnostic and therapeutic aspects as an aid to clinicians and scientists alike. Given the unique nature of each of the hormones, it stands to reason that it will need dynamic conditions for every one of the hormones to be measured and studied. It requires clinical experience and expertise to correctly interpret the hormones and bring in the value addition to patient care.

Having a handbook such as this well-collated dynamic endocrine tests is an asset especially when it is born in the hands of experienced clinicians like Dr Sanjay Bhadada and his team with rich legacy of clinical wealth that has time and again allowed to look back and validate the correct interpretation of these tests.

This is indeed a need of the hour for all clinicians alike, and having it on the go is extremely helpful. In addition to the tests as well as their detailed execution and interpretation, there are clinical scenarios used aptly throughout the book to drive home the points.

The Department of Endocrinology at PGI Chandigarh has had a rich legacy of valuable clinical workload and stalwarts of faculties to correctly apply the knowledge. Dr Sanjay Bhadada continues to hold the department's traditional stronghold on science and its application and has taken the next step to correctly narrate the experience to aid all the fellow endocrinologists.

I must congratulate Dr Bhadada and his very able team members for bringing in this well-compiled narrative of clinical value for all the endocrinology colleagues. Indeed it is a huge task to put it all together in a practical manner and produce this handbook. I am sure this book will continue to be a friendly guide to all of us.

Nalini Shah

Preface

"*A journey of a thousand miles begins with a single step.*" *An important step in endocrine diagnosis is the precise manner of performing and interpreting dynamic tests.*

The basic tenet of clinical testing in endocrinology rests on the paradigm of hormone-feedback loop. Assessment of hormone deficiency requires stimulation and hormone excess entails suppression of the corresponding endocrine axis. Though the basic principle underlying these protocols is dynamic testing, there are certain nuances of the various tests that render them unique. This book is an effort to collate the basic principles, methods, and details of the various tests used in endocrinology. There is no single source or collection of these tests in the country or, to the best of our knowledge, in the south-east Asia region. Hence, we aimed to provide a single document to serve as a useful clinical source of these tests.

Testing protocols in endocrinology are integral to the diagnosis of various endocrine conditions. The basic pillar of diagnosis in endocrinology is to first demonstrate the hormone dysfunction using biochemical tests and only then proceed to imaging. Dynamic tests also play a pivotal role in follow-up and management, in terms of assessment of response to therapy. The book attempts to compile various facets of dynamic tests, in a systematic fashion, pertaining to the pituitary, thyroid, bone, pancreas, adrenals, and gonads. Testing for acromegaly, Cushing's syndrome, and primary hyperparathyroidism in special situations such as pregnancy and chronic kidney disease has also been addressed. Procedures such as the inferior petrosal sinus sampling and the adrenal venous sampling have been elucidated. Each of these tests has been organized under the headings of principle, indications, contra-indications, procedure, and pitfalls. Possible complications of each test and troubleshooting measures have been mentioned, wherever applicable. The format is user-friendly, and the language has been kept lucid, making efforts to provide references or derive from the vast experience of the department.

The purpose of this book is to provide a "ready reckoner" for the various dynamic tests in endocrinology to be used at the bedside. We hope this serves as a useful source of information for students, physicians, and endocrinologists alike.

Chandigarh, India — Sanjay K. Bhadada
Chandigarh, India — Liza Das
Chandigarh, India — Rimesh Pal

A L G r a w a n y

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Contents

1 **Endocrinology Evaluation Protocol: Pituitary and Hypothalamus** 1
Rahul Gupta, Liza Das, and Pinaki Dutta

2 **Endocrinology Evaluation Protocol: Diabetes and Thyroid** 41
Satyam Singh Jayant, Rimesh Pal, and Sanjay Kumar Bhadada

3 **Endocrinology Evaluation Protocol: Adrenals** 63
Kanhaiya Agrawal, Soham Mukherjee, and Rama Walia

4 **Endocrinology Evaluation Protocol: Gonads** 93
Shakun Chaudhary, Ashu Rastogi, and Rama Walia

5 **Endocrinology Evaluation Protocol: Bone and Mineral Disorders** . . . 105
Rimesh Pal, Sanjay Kumar Bhadada, and Pinaki Dutta

6 **Endocrinology Evaluation Protocol: Special Situations** 115
Shakun Chaudhary, Liza Das, and Soham Mukherjee

A L G r a w a n y

About the Editors

Sanjay Kumar Bhadada is currently working as Professor and Head of the Department of Endocrinology at the Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh. He has more than 350 peer-reviewed international publications and has delivered numerous oral and poster presentations in multiple international meetings. He is the President of the Endocrine Society of India and RSSDI (Punjab Chapter). He is on the editorial board of reputed journals, such as the Journal of Clinical Endocrinology and Metabolism, Calcified Tissue International, and Journal of Endocrinological Investigation and has contributed a book chapter in the William's textbook of Endocrinology and The Parathyroids. He is also the principal investigator of the Indian PHPT Registry and the Rare Metabolic Bone Disease Registry. He is selected to receive the US Endocrine Society's highest Laureate award, the Outstanding Mentor award.

Liza Das is a clinical endocrinologist working at the Department of Endocrinology, Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh. She has to her credit over 60 peer-reviewed publications in acclaimed journals, like Journal of Clinical Endocrinology and Metabolism, Diabetes Care, Frontiers in Endocrinology, Pituitary, and Journal of Endocrinological Investigation. Her work also includes book chapters in national textbooks about diabetes, pituitary gland, and COVID-19. She serves on the editorial board of prestigious journals, such as Frontiers in Endocrinology and PLOS One, and is a reviewer for international journals. Apart from presentations at the US Endo, British Endocrine Society, and the international webinar in pituitary medicine (Endotext), she was recently invited for her guest lecture at the US Endo, 2022, in the Pituitary Symposium. She is the recipient of the David L. Kleinberg Early Career Pituitary Investigator Award by the Pituitary Society US and the Prof. R. Nath gold medal for best research work by PGIMER.

Rimesh Pal is presently working as an Assistant Professor in the Department of Endocrinology at the prestigious Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, India. He has more than 100 peer-reviewed publications in renowned international and national journals, including The Lancet Diabetes & Endocrinology, Nature Reviews Endocrinology, Diabetes Research and Clinical Practice, Osteoporosis International, Frontiers in Endocrinology, Journal of Endocrinological Investigation, and Indian Journal of Medical Research, among others. He is a regular reviewer for multiple international and national journals. Besides, he is an editor of multiple international journals, notably the Therapeutic Advances in Endocrinology and Metabolism, Journal of Osteoporosis, Endocrine and Metabolic Science, Frontiers in Endocrinology: Bone Research, and PLOS One. He was the recipient of the distinguished C. Wayne Bardin International Travel Award for the year 2022, which is awarded by the Endocrine Society to only one endocrinologist globally.

Endocrinology Evaluation Protocol: Pituitary and Hypothalamus

1

Rahul Gupta, Liza Das, and Pinaki Dutta

Contents

1.1 Insulin-Induced Hypoglycaemia (IIH)/Insulin Tolerance Test. 4
1.1.1 Principle. 4
1.1.2 Indications. 4
1.1.3 Contraindications. 4
1.1.4 Preparation. 4
1.1.5 Method. 5
1.1.6 Side Effects. 6
1.1.7 Interpretation. 6
1.2 Clonidine Stimulation Test. 9
1.2.1 Principle. 9
1.2.2 Indication. 9
1.2.3 Contraindications. 9
1.2.4 Preparation. 9
1.2.5 Method. 9
1.2.6 Side Effects. 10
1.2.7 Interpretation. 10
1.2.8 Interpretation. 10
1.3 Glucagon Stimulation Test. 10
1.3.1 Principle. 10
1.3.2 Indication. 10
1.3.3 Contraindications. 10
1.3.4 Preparation. 11
1.3.5 Method. 11
1.3.6 Side Effects. 11
1.3.7 Interpretation. 11
1.4 Levodopa Stimulation Test. 11
1.4.1 Principle. 11

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A L G r a w a n y

1.4.2 Indication..... 12
1.4.3 Contraindications..... 12
1.4.4 Preparation..... 12
1.4.5 Method..... 12
1.4.6 Side Effects..... 13
1.4.7 Interpretation..... 13
1.5 IGF-1 Generation Test..... 13
1.5.1 Principle..... 13
1.5.2 Indication..... 13
1.5.3 Method..... 14
1.5.4 Interpretation..... 14
1.5.5 CEMRI Sella..... 18
1.5.6 Interpretation..... 18
1.6 Oral Glucose Tolerance Test for Acromegaly..... 18
1.6.1 Principle..... 18
1.6.2 Indication..... 18
1.6.3 Contraindication..... 18
1.6.4 Method..... 19
1.6.5 Side Effects..... 19
1.6.6 Interpretation..... 19
1.7 Bilateral Simultaneous Inferior Petrosal Sinus Sampling (BIPSS)..... 20
1.7.1 Principle..... 20
1.7.2 Indication..... 20
1.7.3 Contraindications..... 20
1.7.4 Preparation..... 21
1.7.5 Method..... 22
1.7.6 Side Effects..... 23
1.8 CRH Testing..... 26
1.8.1 Indication..... 26
1.8.2 Contraindications..... 26
1.8.3 Method..... 26
1.8.4 Side Effects..... 27
1.8.5 Interpretation..... 27
1.9 IV AVP Stimulation Test..... 27
1.9.1 Indication..... 27
1.9.2 Contraindications..... 27
1.9.3 Method..... 27
1.9.4 Side Effects..... 28
1.9.5 Interpretation..... 28
1.9.6 Interpretation..... 29
1.10 Low-Dose Dexamethasone Suppression Test (LDDST)..... 29
1.10.1 Principle..... 29
1.10.2 Indication..... 29
1.10.3 Contraindications..... 29
1.10.4 Method..... 29
1.10.5 Interpretation..... 30
1.11 High-Dose Dexamethasone Suppression Test (HDDST)..... 30
1.11.1 Indication..... 30
1.11.2 Contraindications..... 30
1.11.3 Method..... 30
1.11.4 Interpretation..... 31
1.12 Water Deprivation Test..... 31
1.12.1 Indication..... 31
1.12.2 Prerequisites..... 31

1.12.3 Contraindications..... 32
1.12.4 Preparation..... 32
1.12.5 Method..... 32
1.12.6 Side Effects..... 35
1.13 Prolonged Water Deprivation Test..... 35
1.13.1 Indication..... 35
1.13.2 Methods..... 36
1.13.3 Interpretation..... 36
1.14 Patient Instructions Sheet..... 37
1.14.1 Instructions to the Patient for Dexamethasone Suppression Test..... 37
1.15 Instructions to the Patient for Urine Collection for 24-h Urinary Free Cortisol Test..... 38
1.16 Instructions to the Patient for 11 pm Awake Cortisol and ACTH..... 38
Suggested Reading..... 39

Abbreviations

ACTH	Adrenocorticotrophic hormone
AVP	Arginine vasopressin
BIPSS	Bilateral inferior petrosal sinus sampling
CDI	Central diabetes insipidus
CRH	Corticotrophin-releasing hormone
CVA	Cerebrovascular accident
DDAVP	Desmopressin
ECG	Electrocardiogram
EDTA	Ethylenediamine tetraacetic acid
GH	Growth hormone
GHD	Growth hormone deficiency
GHRH	Growth hormone-releasing hormone
HbA1c	Glycated haemoglobin
HDDST	High-dose dexamethasone suppression test
IGF-1	Insulin-like growth factor 1
IIH	Insulin-induced hypoglycaemia
IJV	Internal jugular vein
INJ	Injection
INR	International normalized ratio
IV	Intravenous
LDDST	Low-dose dexamethasone suppression test
L-DOPA	Levodopa
NDI	Nephrogenic diabetes insipidus
NPO	Nil per orally
ONDST	Overnight dexamethasone suppression test
PO	Per orally
PP	Primary polydipsia
S.OSMO	Serum osmolality
TV	Testicular volume

A L G r a w a n y

U.OSMO Urine osmolality
UFC Urinary free cortisol
UO Urine output

1.1 Insulin-Induced Hypoglycaemia (IIH)/Insulin Tolerance Test

1.1.1 Principle

Hypoglycaemia induces an increase in GHRH and CRH secretion.

1.1.2 Indications

- Assessment of growth hormone reserve in children and adults
- Assessment of ACTH and cortisol reserve
- Differentiation of Cushing's syndrome from pseudo-Cushing's state

1.1.3 Contraindications

- Ischemic heart disease (baseline ECG in cases when clinically suspected), cardiac rhythm disorders, prior cerebrovascular accident
- Epilepsy
- Untreated hypothyroidism

1.1.4 Preparation

(a) Minimum basic investigations required are IGF-1, serum 8 am cortisol, thyroid, liver and renal function tests.
(b) Need for sex hormone priming (Table 1.1):

Table 1.1 Methods of priming prior to testing for GH reserve

Drug	Dose
Conjugated equine oestrogen (Premarin)	Total 10 mg dose divided into 5 mg PO (0.625 mg × 8 tablets) in the previous night and 5 mg in the morning of the test
Oestradiol valerate	2 mg PO for 3 consecutive days (>20 kg body weight) 1 mg PO for 3 consecutive days (<20 kg body weight)
Testosterone enanthate	100 mg IM injections 3 day prior to test

Conjugated equine oestrogen and oestradiol valerate can be used in both boys and girls, while testosterone is only for boys

  - Indicated in prepubertal males (TV <4 cc) of more than 11 years of chronological age and prepubertal females (<B2) of more than 10 years of chronological age.
  - Regimen (tick mark whichever is appropriate).

1.1.5 Method

- The patient should be fasting overnight (water permitted) and be recumbent during the test.
- Ongoing medication can be given after completion of the test, i.e. by lunchtime. If you simultaneously want to assess GH and cortisol reserve and patient is taking hydrocortisone, then the last dose should be at midday, the day before the test (i.e. omit evening dose and dose on morning of test). If only GH reserve is to be assessed, steroid supplement should continue on the day of the test as usual.
- The test should be supervised at all times.
- Ensure availability of all materials (see consent form) required for the test and availability of emergency cart.
- Two separate IV lines should be secured and patency ensured by frequent heparinized saline flush [made by dissolving 25,000 U of heparin (1 vial) in 500 mL of normal saline].
- Take baseline (t = 0 min) blood sample for GH, cortisol (purple-top vial) and glucose (grey-top vial) and check plasma glucose using glucometer.
- Calculate regular insulin dose: 0.10 U/kg.
- The calculated insulin dose should be withdrawn from the vial using insulin syringe. This should be reconstituted with normal saline to make a total volume of 1 mL (fill the U40 insulin syringe to 40 U mark with normal saline). The same should be transferred to 2 mL syringe, reconstituted to 2 mL using normal saline and given as an IV bolus followed by flush with 5 mL normal saline.
- Check blood glucose with glucometer every 15 min until adequate hypoglycaemia is achieved (<40 mg/dL). Testing for glucose can be done earlier than 15 min in case of hypoglycaemic symptoms. In case adequate hypoglycaemia is not achieved even after 30 min of IV insulin administration, repeat the IV insulin dose at 0.15–0.2 IU/kg.
- Adequate hypoglycaemia (<40 mg/dL) should be achieved, and associated symptoms should be recorded. The time of attainment of hypoglycaemia is considered as "t= 30 min" from baseline (this stands irrespective of the actual time taken for achieving hypoglycaemia in relation to baseline sample). At this time (t = 30), blood sample should be drawn for GH, cortisol (purple-top vial) and blood glucose (grey-top vial).
- Subsequently take sample at t = 60, 90 and 120 min for GH and cortisol (purple-top vial), flushing the cannula with heparinized saline between samples. Check blood glucose on glucometer every time a specimen is taken.

A L G r a w a n y

- After attainment of adequate hypoglycaemia, patient should be allowed oral feeds (200–300 mL of carbonated beverage or fresh juice), and resolution of hypoglycaemia should be documented after 15 min.
- Once test is completed, give supervised meal.
- Monitor patient for 2 h after completion of the test.

1.1.5.1 Troubleshooting

- If symptoms of hypoglycaemia are very severe, or the patient is unarousable (rare), or hypoglycaemia lasts for more than 30 min after oral feed, or in case of vomiting immediately after oral feed, consider giving IV 25% dextrose (25–50 mL) and continue sampling. Overcorrection of hypoglycaemia with intravenous dextrose needs to be avoided.
- If a patient develops adrenal crisis (hypotension), they should receive IV 0.9% saline and IV hydrocortisone 100 mg.
- Keep dextrose 25% and prefilled syringe of 100 mg hydrocortisone available beforehand.

1.1.6 Side Effects

Some common side effects are sweating, palpitations and rarely loss of consciousness and convulsions.

1.1.7 Interpretation

- Adult GHD: peak growth hormone level <5.1 ng/mL suggests growth hormone deficiency (sensitivity: 96%, specificity: 92%).
- Childhood GHD: peak growth hormone level <10 ng/mL suggests growth hormone deficiency.
- Adrenocortical reserve: peak plasma cortisol >550 nmol/L suggests adequate adrenocortical function.
- Cushing vs. pseudo-Cushing.

Case 1: Insulin-Induced Hypoglycaemia

This is a case of a 14-year-old boy who presented with short stature and absent puberty (Fig. 1.1). Short stature was noticed at the age of 5 years. There was no history to suggest chronic systemic illness, intracranial mass or malabsorption. His birth weight was 3 kg, and he had prolonged neonatal jaundice and history of hypoglycaemia post-birth but no further episodes of hypoglycaemia. Also, developmental milestones were normal, and scholastic performance was average. On examination, his height was 118 cm, weight 20 kg and BMI 14.36 kg/m^2. His upper

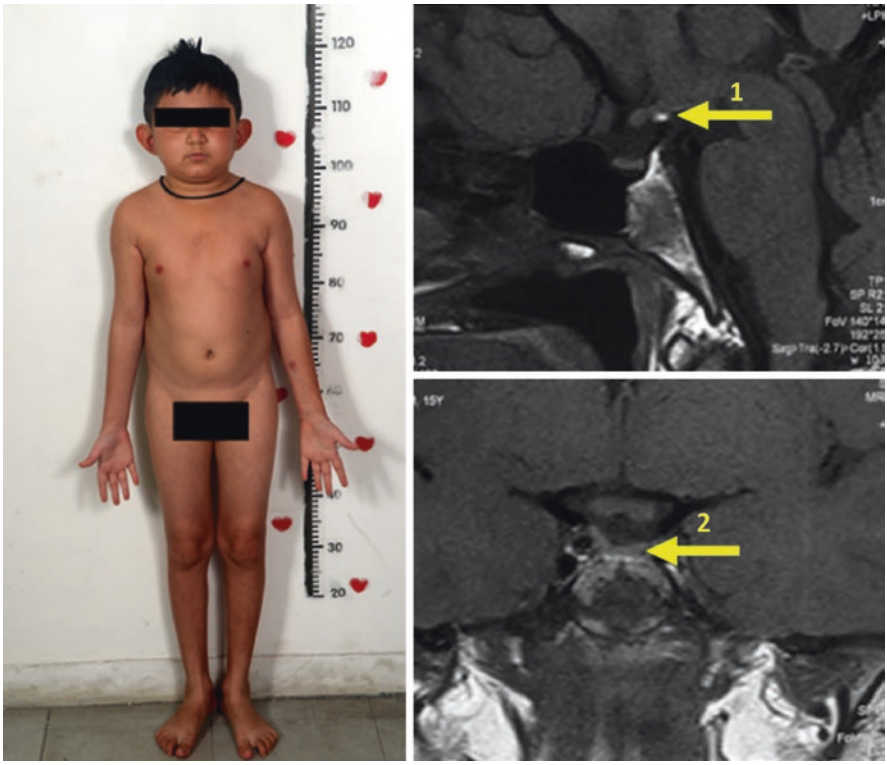

Fig. 1.1 Panel of photographs showing the boy with short stature and MRI depicting the classical features of small sella, hypoplastic pituitary gland, redundant stalk and ectopic posterior pituitary bright spot (1. Ectopic posterior pituitary bright spot 2. Small sella and hypoplastic anterior pituitary gland)

segment-to-lower segment ratio was 0.87. His height SDS was −5.4. At a chronological age of 14 years, his bone age was 8 years and height age was 6 years. His sexual maturity rating was Tanner 1 with testicular volume of 1 mL bilaterally and stretched penile length of 3.5 cm. He had a cherubic face. Visual acuity and visual fields were normal. There were no midline or skeletal defects, and systemic examination was normal. In view of severe short stature and markedly delayed height age, bone age and a positive history of hypoglycaemia, a possibility of multiple pituitary hormone deficiency, including growth hormone deficiency, was kept. His baseline complete blood counts and renal and liver function tests were within normal limits, and celiac serology was negative. IGF-1 was low as was 8 am cortisol and total T4 was normal on levothyroxine replacement (Table 1.2). To confirm the presence of growth hormone deficiency, we went ahead and performed insulin-induced hypoglycaemia (Table 1.3) and clonidine stimulation tests (Table 1.4).

A L G r a w a n y

Table 1.2 Hormonal profile of case 1

Hormone	Values
GH basal (ng/mL)	0.459
IGF-1 (ng/mL)	**<15 (211–512)**
TSH (uIU/mL)	0.006
T3 (ng/mL)/T4 (μg/dL)	1.03/8.46
FT3 pmol/L	3.85 (3.1–6.8)
FT4 pmol/L	6.98 (12–22)
LH (mIU/L)	0.696
FSH (mIU/L)	0.751
PRL (ng/mL)	8.51
Testosterone (nmol/L)	**0.0868**
S. cortisol 8 am (nmol/L)	**5.92**
ACTH 8 am (pg/mL)	1.50
DHEAS (μg/dL)	0.2

Table 1.3 Results of growth hormone stimulation test (insulin-induced hypoglycaemia)

Time (min)	Random plasma glucose (mg/dL)	GH (ng/mL)
0	77	0.24
30	22	0.14
60	68	0.11
90	80	0.09
120	85	0.08

Table 1.4 Results of growth hormone stimulation test (clonidine simulation test)

Time (min)	GH (ng/mL)
0	0.638
30	0.428
60	0.718
90	0.720
120	0.8

As he was found to be hypothyroid and hypocortisolic and was more than 12 years of age, the test was performed after making the child euthyroid, eucortisolic and priming with conjugated equine oestrogen. Morning dose of hydrocortisone was given before the test.

The peak GH was subnormal (<10 ng/mL), suggesting growth hormone deficiency.

1.2 Clonidine Stimulation Test

1.2.1 Principle

Clonidine induces GHRH secretion via alpha-2 receptor activation.

1.2.2 Indication

Assessment of growth hormone reserve in children with suspected growth hormone deficiency.

1.2.3 Contraindications

- No absolute contraindication.
- Negative consent.

1.2.4 Preparation

(a) Minimal basal investigations required are IGF-1, serum cortisol, thyroid function test, liver function test and renal function test.
(b) Need for sex hormone priming: same as in IIH test.

1.2.5 Method

- Test should be performed in the morning after an overnight fast; however, drinking water is allowed.
- Medications to be taken on time with a sip of water.
- Test to be performed in a recumbent position.
- Ensure availability of all materials (see consent form) required for the test and that of emergency cart.
- Insert an IV butterfly cannula and then rest for 30 min prior to −15-min sample. Ensure patency by heparinized saline flush after every sampling [made by dissolving 5000 U of heparin (1 vial) in 500 mL of normal saline].
- Oral clonidine 0.15 mg/m^2 body area is given at time, $t = 0$.
- Take peripheral blood sample in purple-top vials for GH at: –15′…0′…15′…30′…45′…60′…90′…120′
- Monitor blood pressure and heart rate every 30 min.
- Patient should be at rest for 30 min after the procedure. Check blood pressure and postural drop before one is allowed to leave.

A L G r a w a n y

1.2.6 Side Effects

Some common side effects are drowsiness which may persist for 2–3 h, dry mouth, dizziness, nausea and headache and rarely a fall in blood pressure.

1.2.7 Interpretation

- Peak GH levels of >10 ng/mL indicate adequate growth hormone reserve.

Case 1: Clonidine Stimulation Test
The same patient (as mentioned in the insulin-induced hypoglycaemia test) underwent clonidine test (a second stimulation test) to confirm GHD. MRI later revealed absent stalk, ectopic PPBS and small sella.

1.2.8 Interpretation

GH value less than 10 ng/mL suggests growth hormone deficiency, and all values less than 5 suggest severe GHD.

1.3 Glucagon Stimulation Test

1.3.1 Principle

Exact mechanism of glucagon-induced growth hormone secretion is not known. However, one of the postulated mechanisms is the presence of putative peptides in glucagon preparation that act as growth hormone secretagogues.

1.3.2 Indication

Assessment of growth hormone reserve in children and adults with suspected growth hormone deficiency.

1.3.3 Contraindications

- Suspected intestinal obstruction or pheochromocytoma.
- Known hypersensitivity reaction to glucagon.
- Negative consent.

1.3.4 Preparation

(a) Minimal basal investigations required are IGF-1, serum cortisol, thyroid function test, liver function test and renal function test.
(b) Need for sex hormone priming: same as in IIH test.

1.3.5 Method

- Test should be performed in the morning after an overnight fast.
- Medications should however be taken on time with a sip of water.
- Ensure availability of all materials (see consent form) required for the test and that of emergency cart.
- Insert an IV cannula and ensure patency by heparinized saline flush after every sampling [made by dissolving 25,000 U of heparin (1 vial) in 500 mL of normal saline].
- Calculate glucagon dose: adults: 1 mg, (1.5 mg if >90 kg); children: 30 mcg/kg.
- Administer the calculated glucagon dose intramuscularly.
- Take peripheral blood sample in purple-top vials for GH at time, *t*=: 0′…30′… 60′… 90′… 120′… 150′… 180′… 240′
- Monitor blood pressure and heart rate every 30 min and document any side effects.

1.3.6 Side Effects

- Some common side effects are nausea, vomiting, abdominal pain and allergic reaction.

1.3.7 Interpretation

- Peak GH levels of >10 ng/mL indicate adequate growth hormone reserve.
- Peak GH <3 ng/mL in adults indicates GH deficiency. (as per the Growth Hormone Research Society and Endocrine Society).

1.4 Levodopa Stimulation Test

1.4.1 Principle

L-dopa-mediated increase in norepinephrine levels in limbic system, which in turn increases dopamine levels in median eminence causing release of GHRH.

A L G r a w a n y

1.4.2 Indication

Assessment of growth hormone reserve in children and adults with suspected growth hormone deficiency.

1.4.3 Contraindications

- Coronary artery disease, cardiomyopathy, psychiatric comorbidity.
- Negative consent.

1.4.4 Preparation

(a) Minimal basal investigations required are IGF-1, serum cortisol, thyroid function test, liver function test and renal function test.
(b) Need for sex hormone priming: same as in IIH test.
(c) Levodopa: L-dopa is only available in combination with carbidopa. The commercially available preparations have L-dopa/carbidopa ratio of 10:1 and is usually available as 110 mg tablet (100 mg levodopa/10 mg carbidopa) (Table 1.5).

1.4.5 Method

- Test should be performed in the morning after an overnight fast; however, drinking water is allowed.
- Medications to be taken on time with a sip of water.
- Test to be performed in a recumbent position.
- Ensure availability of all materials (see consent form) required for the test and that of emergency cart.
- Insert an IV cannula and then rest for 30 min prior to −15-min sample. Ensure patency by heparinized saline flush after every sampling [made by dissolving 25,000 U of heparin (1 vial) in 500 mL of normal saline].
- Give the calculated dose of levodopa at time, $t = 0$.
- Take peripheral blood sample in purple-top vials for GH at: 0′ …30′… 60′… 90′… 120′
- Monitor blood pressure and heart rate every 30 min.

Table 1.5 Dosing regimens for levodopa stimulation testing

Body weight (kg)	L-dopa (mg)	L-dopa/carbidopa ratio of 10:1 (tablets)
<13.5	125	1.25
13.5–31.5	250	2.5
>31.5	500	5

- Patient should be at rest for 30 min after the procedure. Check blood pressure and postural drop before one is allowed to leave.

1.4.6 Side Effects

- Some common side effects are nausea, vomiting and hypotension.

1.4.7 Interpretation

- Peak GH levels of >10 ng/mL indicate adequate growth hormone reserve in children.
- Peak GH level of >1.1 ng/mL indicates adequate growth hormone reserve in adults.

Other growth hormone stimulations that are in common use include arginine stimulation test, combined arginine and levodopa stimulation test, growth hormone-releasing hormone test, combined growth hormone-releasing hormone-arginine test and macimorelin test. However, due to non-availability of these compounds, they are currently not being performed at our centre.

1.5 IGF-1 Generation Test

1.5.1 Principle

IGF-1 generation is GH dependent at all ages. The aim of this test is to differentiate GH insensitivity (GHI) from bioinactive GH.

1.5.2 Indication

The test is indicated in cases of short stature meeting the below-mentioned auxologic criteria, with peak GH level >15 μg/L and GHBP (<−2SD):

- Severe short stature (height SDS <−3 SD)
- Severe growth deceleration (height velocity SDS <−2 SD over 12 months)
- Height <−2 SD and height velocity <−1.0 SD over 12 months
- Height <−1.5 SD and height velocity <−1.5 SD over 2 years

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1.5.3 Method

1.5.3.1 Standard 5 Day IGF-1 Generation Test

- Blood samples are obtained for IGF-1 prior to the test, in fasting state in the morning.
- hGH is administered daily for 4 days subcutaneously at a dose of 33 μg/kg/day.
- IGF-1 sampling is done 12 h after the last GH injection, on day 5.

All samples are placed on ice, immediately centrifuged in a refrigerated centrifuge and stored at −70 °C until tested.

1.5.4 Interpretation

IGF-1 increment less than twice the intra-assay coefficient of variation (2 × CV) or an absolute IGF-1 increment of 15 ng/mL indicates GH sensitivity.

1.5.4.1 7-Day IGF-1 Generation Test

- After drawing a baseline serum IGF-1 sample, either a low dose (0.025 mg/kg/d) or a high dose (0.05 mg/kg/d) of GH is given for 7 days.
- Subjects were taught to self-administer SC injections of GH, which were given every evening.
- Fasting blood samples are drawn on the mornings of days 1, 5 and 8.

Poor responders: inability to generate IGF-1 levels above 100 ng/mL on either day 5 or day 8 of the treatment week.

1.5.4.2 2-Week IGF-1 Generation Test

- 3 steps of 2-week SC hGH treatment:
  - Step 1: 0.7 mg/m^2/day
  - Step 2: 1.4 mg/m^2/day
  - Step 3: 2.8 mg/m^2/day
- IGF-1 and IGFBP3 to be measured at the start and end of each step.
- 4-Week washout between steps and review of results.
- Proceed to the next step if increase in IGF-1 <1 SD in the previous step.

Case 2: IGF-1 Generation Test

This is a 22-year-old boy who presented at 13.5 years with growth faltering since infancy (Fig. 1.2). His postnatal period was uneventful. Developmental milestones were normal, and scholastic performance was good. He had immature cherubic face, micrognathia, low-set ears, short neck (neck:height = 1:10) and prepubertal voice. Anthropometry:height SDS was −12 (95 cm) and weight SDS −7 (13 kg), at mid-parental height of 170 cm (Table 1.6). His bone age was 8 years, and height age was 3 years. His sexual maturity rating was A-P1 testicular volume of 4–6 mL

Fig. 1.2 Photograph showing the 22-year-old male who presented with severe short stature and a biochemical profile revealing low IGF-1 but stimulable GH on GH stimulation tests, with good response to recombinant growth hormone, consistent with a diagnosis of bioinactive GH

bilaterally and stretched penile length of 3 cm. Workup for chronic systemic illness was negative. He was being managed with l-thyroxine from outside. Hormonal evaluation was suggestive of low IGF-1 and IGFBP-3 (Table 1.7). Hence, GH stimulation tests were performed which showed peak GH levels >15 ng/ml (Table 1.8). Thus, in view of phenotypic features, very severe short stature and high GH on provocative testing, a possibility of GH resistance was considered and IGF-1 generation test was performed, which did not show adequate increment in IGF-1 (Table 1.9). However, he continued to show response to rhGH treatment, with an increase in height velocity (Tables 1.10 and 1.11). Hence, IGF-1 generation was repeated on follow-up at 18 years of age, which showed 55% increment at day 7,

Table 1.6 Anthropometry parameters of case 2

Weight	13 kg
Wt. age	3 years
Wt. SDS	**−7**
Height	95 cm
Ht. age	2.5 years
Ht. SDS	**−12**
Upper segment/lower segment	46 cm/49 cm = 0.93
Chronological age	13.5 years
Bone age	8 years
Height age	3 years
CA > BA > HA	
Tanner stage	A-P1TV: 4–6 mL (b/L); SPL = 3 cm

Table 1.7 Hormonal profile of case 2

IGF-1	**<25 ng/mL (low)**
IGFBP-3	**<0.5 µg/mL (3.5–10) (low)**
T4 (µg/dl)	8.23
TSH (uIU/ml)	2.28
LH (mIU/L)	0.44
FSH (mIU/L)	1.17
Testosterone (nmol/L)	0.82
DHEAS (µg/mL)	316
HbA1C	5.7%

Table 1.8 GH stimulation tests (after priming)

	IIH	Clonidine stimulation test
	GH (ng/mL)	GH (ng/mL)
0	3.13	1.50
30 min	1.71	1.23
60 min	16.82	17.43
90 min	10.28	42.95
120 min	21.18	

Table 1.9 IGF-1 generation test of case 2

Baseline IGF-1	<25 ng/mL
After GH (@0.033 mg/kg)	<25 ng/mL

consistent with a diagnosis of bioinactive GH. He achieved a final height of 125 cm after 6 years of GH treatment. For delayed puberty, he received testosterone injections monthly for 6 months, after which he spontaneously entered into puberty, and hence injection testosterone was stopped.

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Table 1.10 Response of case 2 to recombinant human growth hormone (hGH) treatment

	March 13	July 13	November 13	January 14	July 14	July 15	October 15	December 16	June 17	July 19
Height (cm)	92	93	94	98	102	108.2	111	116	121	125
Weight (kg)	13.6	15	15	15.5	16.5	20.5	20	30	34	37.5

Table 1.11 Height gain in case 2 following growth hormone treatment

On GH treatment	Height gain (cm)
1st year	9
2nd year	6.2
3rd year	6.5
4th year	7
5th year	3
6th year	2

1.5.5 CEMRI Sella

Pituitary stalk is in midline. Sella is empty and filled with CSF. Pituitary bright spot is not visualized. Final impression: empty sella.

1.5.6 Interpretation

In the index patient (case 2), IGF-1 generation test (5-day testing) was performed, which showed an IGF-1 (after GH administration) to be non-stimulable (<25 ng/mL). This suggested a negative IGF-1 generation.

1.6 Oral Glucose Tolerance Test for Acromegaly

1.6.1 Principle

- Hyperglycaemia-mediated increased somatostatin tone leading to inhibition of growth hormone secretion.

1.6.2 Indication

- Clinical suspicion of acromegaly with an elevated IGF-1.
- Follow-up of acromegaly cases post-surgery.

1.6.3 Contraindication

- No absolute contraindication.
- Known diabetic with poor glycaemic control.
- Negative consent.

1.6.4 Method

- Test should be performed in the morning after an overnight fast. Medications (routine as per previous prescription, whatever the patient is on) should however be taken on time with a sip of water.
- Ensure availability of all materials (see consent form) required for the test.
- Insert an IV cannula and ensure patency by heparinized saline flush after every sampling [made by dissolving 25,000 U of heparin (1 vial) in 500 mL of normal saline].
- Take blood sample for GH (into a purple-top Vacutainer) and glucose (into grey-top tube) at $t = 0$ min. Simultaneously check plasma glucose using glucometer.
- Administer 75 g anhydrous oral glucose (if not available, then 82.5 g of unflavoured glucose can be used) in 300 mL water over about 10 min.
- Take blood for GH and glucose at $t = 60$ and 120 min.
- In case bedside fasting plasma glucose is more than 200 mg/dL, sample for GH and glucose at $t = 30$, 60, 90 and 120 min can be taken without administration of 75 g glucose.

1.6.5 Side Effects

Some common side effects are nausea, vomiting and abdominal pain.

1.6.6 Interpretation

- A normal response is a nadir growth hormone level of <0.4 ng/mL.

Case 3: Oral Glucose Tolerance Test
A 28-year-old male patient presented with coarse features and acral enlargement (Fig. 1.3). Investigations revealed high IGF-1 (1105 ng/mL, normal 86–284). Oral glucose tolerance test was performed, which showed non-suppressible GH (nadir 55 ng/mL).

1. T1-weighted coronal MRI of the patient showing pituitary macroadenoma (17.8 × 25.2 × 16.7 mm, Knosp 3b) abutting the optic chiasma.

Fig. 1.3 Panel of photographs of the 28-year-old male patient with acromegaly who was found to have high IGF-1 (1105 ng/mL, normal 86–284) and non-suppressible GH on oral glucose tolerance test (nadir GH 55 ng/mL). T1-noncontrast MRI coronal section depicts a pituitary macroadenoma, which was operated by the endoscopic transsphenoidal route, and it revealed an adenoma which was growth hormone and prolactin positive by immunohistochemistry

1.7 Bilateral Simultaneous Inferior Petrosal Sinus Sampling (BIPSS)

1.7.1 Principle

Measurement of ACTH in petrosal sinuses and periphery simultaneously to determine the source of ACTH excess.

1.7.2 Indication

- To differentiate pituitary from a non-pituitary source of ACTH.

1.7.3 Contraindications

- Bleeding or thrombosis tendencies (get a complete blood count, coagulogram and renal function test).
- Orthopnoea.

- Relative contraindications: uncontrolled hypertension, ischemic heart disease, uncontrolled sepsis, hypokalaemia, deep vein thrombosis, inability to stop ketoconazole/mifepristone therapy (all of them need to be stopped 1 week before BIPSS), use of anticoagulants or antiplatelets and renal dysfunction.
- Allergy to contrast dye.

1.7.4 Preparation

- Confirmed case of ACTH-dependent Cushing's syndrome ☐Y ☐N
- [ONDST:_____; LDDST:_____; 24-h UFC_____; 2300-h plasma cortisol_____; ACTH_____] IV ADH/CRH:; HDDST:
- MRI sella.
- Prior appointment for the procedure should be taken from the department of interventional radiology. (Try for an appointment in the early morning.)
- The patient should be admitted to endocrinology ward at least a day prior to the procedure.
- Consent form, from the endocrinology department and interventional radiology department, should be duly signed and cross-checked.
- Ketoconazole/mifepristone/somatostatin analogues/cabergoline/metyrapone/osilodrostat need to be stopped 1 week before IPSS.
- If patient is on any antiplatelets or anticoagulants, this should be discussed with interventional radiology in advance. If there is a need for stopping them, then due clearance should be sought from the department of cardiology or haematology, as appropriate.
- Agent for stimulation:
  Ovine CRH (100 μg in adults or 60 μg/m^2 in children).
  AVP (5 U, generally available as 1 mL vial containing a total of 20 U).
  * Discuss with the endocrinology and anaesthesia team in advance about the agent to be used.
- The endocrinology lab team should be informed a day prior to the procedure and on the day of the procedure, in order to expedite the sample processing and reporting.
- The day before the procedure, check complete blood count, INR, serum creatinine and electrolytes and plasma glucose. If any abnormality is found, it should be conveyed to the department of intervention radiology.
- Rule out cyclicity by getting an awake 2300 h (a day prior to procedure) and 0800 h cortisol (on the day of procedure). The sample should be sent by 0900 h to the endocrinology lab, and requisition should be sent for immediate processing and reporting. Do not proceed with BIPSS if 2300 h cortisol is <207 nmol/L.
- Patient should be fasting for at least 4 h prior to the procedure.

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- Two persons to assist in sample procedure. Emergency drugs and equipment (to keep injection hyoscine in addition to routine emergency drugs for AVP-induced pain in abdomen) should be available at all times.
- EDTA vials designated for blood sample collection for ACTH estimation should be prechilled by keeping them in fridge overnight. They should be transported in a box lined with ice packs.

1.7.5 Method

1. Communicate with the radiologist regarding how to obtain the peripheral sample. In most cases, the sample can be taken through a separate port within the femoral sheath itself. In cases where such provision is not available (occasionally sheath with narrow lumen may not have such provisions), another peripheral line specifically for sampling should be established. One line should be kept for administering drugs and another for administering ADH. An arterial line should be inserted for continuous BP monitoring while administering ADH. Ensure patency by heparinized saline flush after every sampling [made by dissolving 25,000 U of heparin (1 vial) in 500 mL of normal saline].
2. The process of catheterization will be performed by the department of interventional radiology. The IR team will give a go-ahead clearance for the procedure once there is successful catheterization. In case there is use of IJV sampling from the base of petrosal sinuses, this should be duly recorded.
3. In case of use of AVP as a stimulating agent:
   (a) Obtain basal sample ($t = -6$ min) for ACTH, and prolactin from the three sites (Lt central, Rt central and periphery). The vials should be kept in a box lined with ice pack at all times, until processing.
   (b) Administer 5 U of vasopressin intravenously (dilute 20 U/1 mL of vasopressin in 19 mL normal saline and administer 5 mL over 6 min). Monitor blood pressure, heart rate, rhythm and oxygen saturation at all times.
   (c) Take subsequent simultaneous sample from three sites (Lt central, Rt central and periphery) for ACTH at times 0, 2, 3, 5, 10 and 15 min.
4. In case of use of CRH as a stimulating agent:
   (a) Obtain basal sample ($t = -6$ min) for ACTH, and prolactin from the three sites (Lt central, Rt central and periphery). The vials should be kept in a box lined with ice pack at all times, until processing.
   (b) Administer 100 μg IV CRH at 0 min as IV bolus over 1 min. Monitor blood pressure, heart rate, rhythm and oxygen saturation at all times.
   (c) Take subsequent simultaneous sample from three sites (Lt central, Rt central and periphery) for ACTH at times 0, 2, 3, 5, 10 and 15 min.

*Ensure that at least 3 mL of whole blood is collected per vial. The labelling should be appropriate, and time on vials should be put simultaneously during sampling by the assistant.

Table 1.12 Table depicting the checklist for sampling during the bilateral inferior petrosal sinus sampling procedure

Time	Left central			Right central			Periphery		
	ACTH	Cortisol	Prolactin	ACTH	Cortisol	Prolactin	ACTH	Cortisol	Prolactin
-6 min	✓	-	✓	✓	-	✓	✓	✓	✓
0 min	✓			✓				✓	
2 min	✓			✓				✓	
3 min	✓			✓				✓	
5 min	✓			✓				✓	
10 min	✓			✓				✓	
15 min	✓			✓				✓	

✓ : indicates the timing of sample.

5. The samples should be transferred immediately to the endocrinology lab.
6. The lab ID should be given to samples after they are brought to the lab and entered into the following sheet (Table 1.12).
7. The samples should be processed immediately. In case of anticipated delay, the samples should be centrifuged, plasma separated and stored at −20 degree Celsius in aliquots after proper labelling with lab ID. Post-processing, plasma should be stored at −20 degree Celsius until the results have been fully analysed and discussed with the endocrinology team (reanalysis may be required in cases of unequivocal results).

   ***Do not refrigerate whole blood samples.**
8. Patient should be monitored in ward for the next 24 h. Department of interventional radiology instructions pertaining to ambulation and catheter-site complications should be followed.

1.7.6 Side Effects

- CRH can cause flushing and hypotension, but this is rare with 100 mcg.
- Administration of vasopressin can be associated with hypertension, arrhythmia, chest pain, abdomen pain, nausea and vomiting.

- Other side effects are catheter-site bleeding, haematoma or infection, deep vein thrombosis, pulmonary embolism, IJV thrombosis, subarachnoid haemorrhage, CVA and retro-auricle ache.

The interpretation of BIPSS testing is summarized in Table 1.13.

Case 4: Bilateral Inferior Petrosal Sinus Sampling (BIPSS)
This is a case of a 55-year-old lady who had primary hypothyroidism and hypertension for 5 years and had now presented with 4-month history of proximal myopathy of lower limbs, hyperpigmentation, pedal oedema and deranged glucose records requiring insulin. On evaluation, she had proximal myopathy, hyperpigmentation, osteopenia and hypokalaemia. But there were no striae, cuticular atrophy or other stigmata of Cushing's syndrome. On evaluation, her 8 am cortisol was 1185 nmol/L, and ACTH was 207 pg/mL with a non-suppressible ONDST, LDDST and also non-suppressible HDDST. CEMRI sella was suggestive of microadenoma (5.8 × 6.2 mm) (Fig. 1.4). In view of short history, hypokalaemia, high ACTH and non-suppressible HDDST, a CECT chest and abdomen were also done but did not localize an ectopic source. Hence, a possibility of non-localized ectopic Cushing's syndrome with pituitary incidentaloma vs. Cushing's disease was considered, and IPSS was done. IPSS localized a central source followed by lateralization to the right side (Table 1.14), and based on this, she underwent TSS following which she was in remission with post-operative 8 am cortisol 70 nmol/L. Also, her requirement of anti-diabetics decreased dramatically.

1.7.6.1 Interpretation

For successful catheterization:

Basal prolactin:

- Right central: peripheral = 26.2 (>1.8).
- Left central: peripheral = 5.10 (>1.8).

Table 1.13 Interpretation of the values obtained during the bilateral inferior petrosal sinus sampling procedure

Purpose	Criteria
Successful petrosal sinus catheterization	– A central to peripheral prolactin ratio of >1.8 – ACTH levels >400 pg/mL from respective sinus
ACTH source localization (central-to-peripheral ACTH ratio)	Basal: >2:1 Stimulated: >3:1 Indicates pituitary as a source of excess ACTH
Lateralization in Cushing's disease (inter-sinus ACTH ratio)	>1.4 Indicates lateralization to the side with higher ACTH values

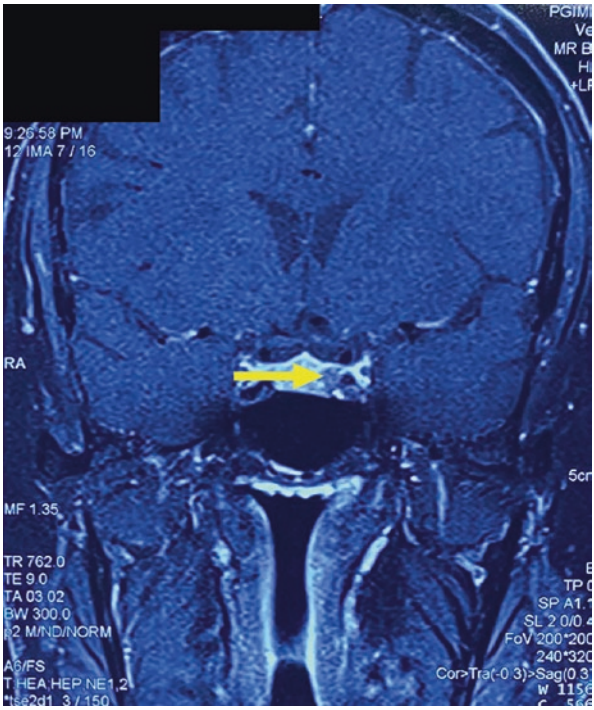

Fig. 1.4 Dynamic contrast-enhanced MRI sella of the patient with ACTH-dependent hypercortisolism depicting a differentially enhancing lesion suggestive of microadenoma (5.8 × 6.2 mm) (yellow arrow) to the left lateral aspect of the pituitary. IPSS was performed in her, which proved localization of the lesion to the pituitary and lateralization to the right

Table 1.14 Results of the BIPSS testing in case 4

	ACTH (right) (in dilution)	ACTH (left)	ACTH (peripheral)	Prolactin (right)	Prolactin (left)	Prolactin (peripheral)
−6 min	4330	357	228	108	21	4.11
0 min	1390	1731	214			
2 min	3700	1384	307			
3 min	**4600**	658	**267**			
5 min	3640	816	248			
10 min	1400	2360 (in dilution)	279			
15 min	2010	1727	253			

The gradient for prolactin is considered only in cases where a central to peripheral gradient for ACTH is not obtained.

For localization:

Basal ACTH:

- Right central: peripheral = (4330/228) = **18.99** (>2).
- Left central: peripheral = (357/228) = 1.56 (< 2).

Stimulated ACTH:

- Right central: peripheral = (4600/267) = **17.22** (>3).

For lateralization:

- Right central: Left central = (4330/357) = **12.12** (>1.4).

1.8 CRH Testing

1.8.1 Indication

- To differentiate pituitary from a non-pituitary source of ACTH in a confirmed case of ACTH-dependent Cushing's syndrome.

1.8.2 Contraindications

- Allergy to ovine CRH.
- Negative consent.

1.8.3 Method

- Test is ideally performed in the morning.
- Ensure availability of all materials (see consent form) required for the test.
- Insert an IV cannula and ensure patency by heparinized saline flush after every sampling [made by dissolving 5000 U of heparin (1 vial) in 500 mL of normal saline].
- Take blood sample for ACTH and cortisol (EDTA vial) at $T = -30$ min. Sample for ACTH to be taken separately in a pre-chilled vial and kept in a box lined with ice packs.
- Patient to remain recumbent throughout the procedure and fasted.
- Two further baseline samples to be taken at −15 min and 0 min for ACTH and cortisol.
- Administer 100 μg human/ovine CRH at $t = 0$, intravenously as a bolus push over 1 min.
- Then take sample for ACTH and cortisol at 15, 30, 45, 60, 90 and 120 min.

1.8.4 Side Effects

- CRH can cause flushing and hypotension, but this is rare with 100 mcg.

1.8.5 Interpretation

- An ACTH increment of more than 35% or cortisol increment of more than 20% is indicative of Cushing's disease. For the purpose of calculations, use the following formula:

$$\text{ACTH increment (\%)} = [\text{Peak ACTH} - \text{ACTH (t0)}] / \text{ACTH (t0)} \times 100.$$

$$\text{Cortisol increment (\%)} = [\text{Peak cortisol} - \text{Cortisol (t0)}] / \text{Cortisol (t0)} \times 100.$$

1.9 IV AVP Stimulation Test

1.9.1 Indication

- To differentiate pituitary from a non-pituitary source of ACTH in a confirmed case of ACTH-dependent Cushing's syndrome.

1.9.2 Contraindications

- No absolute contraindication.
- Relative contraindications: uncontrolled hypertension, cardiac rhythm disorders, heart failure, suspected pheochromocytoma/paraganglioma.

1.9.3 Method

- Test should be performed in the morning after an overnight fast. Medications should however be taken on time with a sip of water.
- Ensure availability of all materials (see consent form) required for the test.
- Insert an IV cannula and ensure patency by heparinized saline flush after every sampling [made by dissolving 25,000 U of heparin (1 vial) in 500 mL of normal saline].
- Take blood sample for ACTH and cortisol (pre-chilled EDTA vial kept in a box lined with ice packs) at $T = -30$ min.
- Patient to remain recumbent throughout the procedure and fasted.

- Two further baseline samples to be taken at −15 min and 0 min for ACTH and cortisol.
- Administer 10 U of AVP (dilute 20 U/1 mL with 4 mL normal saline to make a total volume of 5 mL to make a final concentration of 2.5 U/mL) at $t = 0$.
- Then take sample for ACTH and cortisol at 15, 30, 60 and 90 min.

1.9.4 Side Effects

- Administration of vasopressin can be associated with hypertension, arrhythmia, chest pain, abdomen pain, nausea and vomiting.

1.9.5 Interpretation

- An ACTH increment of more than 35% or cortisol increment of more than 20% is indicative of Cushing's disease. For the purpose of calculations, use the following formula:

$$\text{ACTH increment (\%)} = [\text{Peak ACTH} - \text{ACTH (t0)}] / \text{ACTH (t0)} \times 100.$$

$$\text{Cortisol increment (\%)} = [\text{Peak cortisol} - \text{cortisol (t0)}] / \text{cortisol (t0)} \times 100.$$

Case 4: IV AVP Stimulation Test
In the same patient (mentioned in the IPSS), after ruling out pseudo-Cushing's state, we also performed IV ADH with 5 units of AVP to establish pituitary as the source of ACTH (Table 1.15).

Table 1.15 Results of ADH stimulation test in case 4

	ACTH (pg/mL)	Cortisol (nmol/L)
−30 min	29.1	350
−15 min	33.9	391
0 min	21.4	350
1 min	52.9	385
2 min	**113**	496
5 min	98.5	632
10 min	88.9	800
15 min	94.8	**916**
30 min	70.5	913

1.9.6 Interpretation

258% rise in ACTH (more than 35%) and 147% rise in cortisol (more than 20%) clearly established Cushing's disease in the patient.

1.10 Low-Dose Dexamethasone Suppression Test (LDDST)

1.10.1 Principle

It is based on the principle of differential response of corticotropinoma and normal corticotrophs to dexamethasone. While normal corticotrophs are suppressed by low-dose dexamethasone, corticotropinomas are not.

1.10.2 Indication

- Screening test for suspected endogenous Cushing's syndrome, especially in situations where pseudo-Cushing is a strong consideration.

1.10.3 Contraindications

- Patients on enzyme inducing drugs, e.g. rifampicin and phenytoin.
- Conditions associated with alterations in cortisol-binding globulin: pregnancy, oestrogen therapy, nephrotic syndrome and hypoalbuminemia.
- Oestrogens (e.g. pregnancy, postmenopausal on hormone replacement therapy and combined oral contraceptive).
- Relative contraindications: poorly controlled diabetes mellitus, hypokalaemia, patients with life-threatening psychiatric manifestations, chronic kidney disease and sepsis.

1.10.4 Method

- Can be performed in an inpatient or outpatient setting, provided that the patient is able to understand the instructions clearly and take dexamethasone tablet on time.
- The patient takes 0.5 mg dexamethasone p.o. at *strict* 6-h intervals (i.e. 0900 h, 1500 h, 2100 h and 0300 h) for 48 h. In children weighing <30 kg, dexamethasone dose is 30 μg/kg/day in divided doses.
- The cortisol is measured at 0900 h (before the first dose of dexamethasone) on the first day ("$T = 0$ h") of the test and at 48 h later (6 h after the last dose), i.e. at 0900 h on third day ($T = 48$ h).

- A total of eight doses of dexamethasone should be given in writing to the patient (9 am, then 3 pm, 9 pm, 3 am, 9 am, 3 pm, 9 pm and 3 am). See below for the patient instruction sheet.

1.10.5 Interpretation

- A 0900 h cortisol after 48 h of dexamethasone if >50 nmol/L detects Cushing's syndrome with a sensitivity of 95–97%.
- If the 0900 h cortisol after 48 h is less than 30% from the basal value, one may consider avoiding high-dose dexamethasone suppression test.

1.11 High-Dose Dexamethasone Suppression Test (HDDST)

1.11.1 Indication

To differentiate between pituitary and ectopic source in a patient with ACTH-dependent Cushing's syndrome.

1.11.2 Contraindications

- Patients on enzyme-inducing drugs, e.g. rifampicin and phenytoin.
- Conditions associated with alterations in cortisol-binding globulin: pregnancy, oestrogen therapy, nephrotic syndrome and hypoalbuminemia.
- Oestrogens (e.g. pregnancy, postmenopausal on hormone replacement therapy and combined oral contraceptive).
- Relative contraindications: poorly controlled diabetes mellitus, hypokalaemia, patients with life-threatening psychiatric manifestations, chronic kidney disease and sepsis.

1.11.3 Method

- Can be performed in an inpatient or outpatient setting, provided that the patient is able to understand the instructions clearly and take medicine on time.
- Can follow the LDDST or can be done separately.
- The patient takes 2 mg dexamethasone p.o. at *strict* 6-h intervals (i.e. 0900 h, 1500 h, 2100 h and 0300 h) for 48 h.
- The cortisol is measured at 0900 h (before the first dose of dexamethasone) on the first day ("$T = 0$") of the test and at 48 h later (6 h after the last dose) ($T = 48$). In case where HDDST is preceded by LDDST, the 0800 h cortisol prior to LDDST should be considered as baseline ($T = 0$) and the same should be used for calculation of percent suppression.

Fig. 1.5 Panel of photographs of a 28-year-old female who presented with weight gain and uncontrolled diabetes mellitus. On examination, she had moon facies, supraclavicular fat pad, hirsutism, hyperpigmentation and proximal myopathy. Biochemical tests confirmed an ACTH-dependent hypercortisolism. HDDST was performed in her, which showed 50% suppression from baseline, suggesting pituitary to be the source of Cushing's syndrome

- A total of eight doses of dexamethasone should be given in writing to the patient (9 am, then 3 pm, 9 pm, 3 am, 9 am, 3 pm, 9 pm and 3 am). See below for the patient instruction sheet.

1.11.4 Interpretation

- If the 0900 h cortisol after 48 h is less than 50% from the basal value, it is considered to be suppressed (Fig. 1.5). Suppression on HDDST is more commonly seen in Cushing's disease than in ectopic Cushing's syndrome. (Sensitivity: 65–100%, and specificity: 60–100%)

1.12 Water Deprivation Test

1.12.1 Indication

To distinguish amongst central diabetes insipidus (CDI), nephrogenic diabetes insipidus (NDI) and primary polydipsia (PP) in the evaluation of polyuria.

1.12.2 Prerequisites

1. Confirm polyuria (preferably on an outpatient basis; however, inpatient evaluation can be done in difficult cases) by maintaining a strict input/output diary. Polyuria is defined as a daily urine output of >50 mL/kg/day in adults and 1.5 L/m^2/day in children.

2. Exclude other causes of polyuria: diuretics, chronic renal failure, salt wasting nephropathy, hypercalcaemia, hypokalaemia and hyperglycaemia (baseline investigation should include renal function test, serum electrolytes, urine R/M, urine culture, fasting blood glucose, postprandial glucose, HbA1c, USG KUB).
3. Anterior pituitary hormone deficiencies if present should be adequately replaced.
4. Obtain basal state plasma osmolality, urine osmolality, serum sodium and serum uric acid levels.
5. Osmotic diuresis (urine osmolarity more than 600 mosm/L) should be ruled out prior to conducting the test.

1.12.3 Contraindications

1. If in the basal state plasma osmolality is >295 mosmol/kg or plasma Na >145 meq/L, the test is contraindicated. If along with the above-said criteria, urine is also found to be hypotonic (<300 mosmol/kg), primary polydipsia is excluded and investigation goes straight to AVP administration.
2. Infants.
3. Relative contraindications for the test are prior history of seizure, use of antiepileptics and CVA.

1.12.4 Preparation

1. Patient should be admitted at least a day prior to the procedure.
2. The endocrine lab team should be informed in advance about the need for rapid and multiple estimation of osmolality.
3. The emergency biochemistry lab should be informed in advance about the need for rapid and multiple estimation of serum sodium.
4. The test should be performed under close supervision.
5. The following equipment should be available:
   (a) Digital weighing machine
   (b) Urine pot with volume markings
   (c) 20G/22G IV cannula
   (d) Labelled SERUM (red top) and HEPARINIZED (green top) vials
   (e) 2 mL/5 mL syringe
   (f) Inj vasopressin
   (g) In case one plans to send serum AVP levels, prior coordination should be done with the lab for arranging dry ice and rapid transport of sample.
6. Informed consent

1.12.5 Method

Stage 1 (Exclusion of Primary Polydipsia)

1. Start time should be discussed with the endocrinology team in advance, considering the rate of anticipated dehydration. (For example, in patients with significant polyuria and a basal serum sodium >140 meq/L, water deprivation should begin in the morning. Whereas when the basal serum sodium is <140 meq/L, water deprivation may be initiated at night if it is deemed safe.)
   TROUBLESHOOT
   * However, in scenario where the testing for osmolality and electrolytes is not available at night, it should begin at 0800 h.
2. If the patient is on DDAVP, stop it at least 24 h before the start of the test but continue with other hormone replacement.
3. Insert two IV cannula and ensure patency by heparinized saline flush after every sampling [made by dissolving 5000 U of heparin (1 vial) in 500 mL of normal saline]. Collect baseline samples for serum osmolality (red top vial), urine osmolality (red top vial) and electrolytes (green top vial). The same should be dispatched to endocrinology lab and emergency biochemistry lab for urgent processing.
4. Weigh patient at time 0 and calculate 97% of patient's weight.
5. Urine is passed and discarded at time 0; urine is then passed hourly and volume estimated.
6. Symptoms of thirst and vitals should be recorded every hour.
7. Weight should be measured either every hour or every time 1 L or more of urine has been passed.
8. Subsequently, serum sodium is monitored hourly while serum osmolality and urine osmolality are monitored every two hourly.

All parameters should be entered in a monitoring sheet as depicted in Table 1.16.
End points for stage 1 (if any one of the targets is achieved):

(a) Serum sodium >145 meq/L
(b) Urine osmolarity >700 mOsm/L
   • Two consecutive urine osmolality values differ by no more than 10%.
(c) Loss of >3% body weight
   TROUBLESHOOT
   *In cases where two consecutive urine osmolality values differ by no more than 10% or the patient has lost >3% body weight but serum sodium is <145 meq/L, individual consideration may be given for prolonging the water deprivation for few more hours if it is deemed safe.

Stage 2 (Differential Diagnosis CDI From NDI):

1. Patient may now eat and drink freely.
2. Administer vasopressin 5 U subcutaneously* (available as 20 IU/1 mL vial, dilute with 9 mL normal saline to make a total volume of 10 mL and administer 2.5 mL). If vasopressin is administered, blood pressure and heart rate should be monitored. Empty bladder at the time of injection of vasopressin.

Table 1.16 Table depicting the parameters to be monitored during the water deprivation test

Time	Symptoms	Blood Pressure And Heart Rate	Weight	Urine Output	U.Osmo	S.Osmo	Serum Na

*Dose of vasopressin in children is 1 U/m^2. Avoid desmopressin in children due to risk of water intoxication.

3. Continue to measure hourly urine volumes and osmolality till three consecutive readings do not differ by more than 10%. Drawing blood samples is not indicated, as the patient is now eating and drinking freely, and we are only interested in the effects of the administered DDAVP on urine volume and osmolality.
4. Note down urine volumes in chart.
5. If sodium was >145 meq/L at the completion of stage 1, recheck the values after 2 h to ensure normalization of the same.

The water deprivation test is interpreted based on the results obtained after dehydration and following AVP administration (Table 1.17).

If there is a partial response (300–600 mosmol/L), this test does not reliably differentiate between PP and partial CDI (rarely NDI) as the response to dehydration and DDAVP may be very similar in these two conditions:

1. The post-AVP urine osmolarity response may not be seen in PP or CDI, as polyuria of any origin (e.g. PP or CDI) washes out renal medullary concentration gradient, hence blunting maximal urinary concentration.
2. CDI may increase renal sensitivity to very low levels of AVP. If the patient has only a partial deficiency of AVP, dehydration may therefore rapidly increase urine osmolality to maximum and one may not find any increase post-AVP.
3. Some patients with NDI can concentrate urine if plasma AVP increases to supraphysiological levels, e.g. with exogenous DDAVP.

*If plasma AVP is measured at the end of dehydration, it should be interpreted as follows:

Table 1.17 Interpretation of the water deprivation test. Diagnosis after dehydration and after AVP administration based on urine osmolality values

Diagnosis	Urine osmolality (mosmol/kg) after dehydration (end of stage 1)	Urine osmolality (mosmol/kg) after AVP (end of stage 2)
Normal	>600	>600
PP	300–600	<600
CDI	<300	>600 or ≥50% increase
NDI	<300	<300 or <9% increase

.*Assuming either serum sodium is ≥145 meq/L or serum osmolality ≥300 mosmol/kg

Urine osmolarity at the end of stage 1	Serum Na at the end of stage 1	Urine osmolarity after AVP	Diagnosis
>600	Normal	Not required	Primary polydipsia
<600	>145	>600 or ≥50% increase	Central DI
<600	>145	<300 or <9% increase	Nephrogenic DI
<600	135–145	>9% increase	Central DI
<600	135–145	<9% increase	Primary polydipsia vs. nephrogenic DI

- >1.8 pg/mL: PP
- <1.8 pg/mL: CDI or partial CDI
- *If plasma copeptin is measured at the end of dehydration (serum sodium >145 meq/L), it should be interpreted as follows:
- <4.9 pmol/L: partial CDI (94% sensitivity and 94% specificity)
- >4.9 pmol/L: PP (94% sensitivity and 96% specificity)

To distinguish the partial response, prolonged water deprivation test is required.

1.12.6 Side Effects

Some serious side effects are dehydration and severe hypernatremia leading to seizures, mental obtundation and rarely coma.

1.13 Prolonged Water Deprivation Test

1.13.1 Indication

To distinguish between partial central diabetes insipidus and primary polydipsia after unequivocal water deprivation test.

1.13.2 Methods

- Preparation and methods are similar to water deprivation test, with the only difference being that the patient is kept nil by mouth overnight. The duration of overnight is individualized based on anticipated dehydration rate (findings from water deprivation test can be used for estimating the same). Usually, NPO status is started from 2000 h.
- Weigh patient at time 0, and calculate weight 97% of patient's weight.
- Weight should be measured at hourly intervals: consider stopping test if >3% weight loss (positive test). Monitor UO every two to four hourly.
- Collect urine for osmolality hourly beginning at 0800 h after overnight NPO status. (Samples may be taken and stored if patient loses more than 3% weight and the continuation of test is deemed unsafe.)
- Collect plasma for osmolality and sodium estimation every 2 h beginning at 0800 h after overnight NPO status. (If the patient loses more than 3% of body weight, test can be stopped after taking samples for osmolality and serum sodium.)
- End points for prolonged water deprivation (if any one of the targets is achieved):
  Serum sodium >145 meq/L
  Two consecutive urine osmolality values differ by no more than 10%
  Loss of >3% body weight

*In cases where two consecutive urine osmolality values differ by no more than 10% or the patient has lost >3% body weight and serum sodium is <145 meq/L, individual consideration may be given for prolonging the water deprivation for few more hours if it is deemed safe.

When a plateau in urine osmolality has been reached, administer vasopressin 5 U SC* (available as 20 IU/1 mL vial, dilute it to 10 mL with normal saline and administer 2.5 mL). If vasopressin is administered, blood pressure and heart rate should be monitored.

*Dose of vasopressin in children is 1 U/m^2. Avoid desmopressin children due to risk of water intoxication.

1.13.3 Interpretation

1. **Normal response**: Urine osmolality rises to reach a plateau and does not increase further in response to AVP. Plasma osmolality is maintained within the normal range. Urine osmolality:plasma osmolality ratio is >2 at the end of dehydration.
2. **Primary psychogenic polydipsia***:* Before AVP administration, urine maximum osmolality is >290 mOsm/kg with <9% rise in urine osmolality after AVP. Baseline plasma osmolality is usually low.
3. **Partial CDI:** A rise in urine osmolality of 9% or more after AVP suggests partial cranial DI, i.e. endogenous maximal AVP secretion is insufficient to maximally concentrate the urine.

Case 5: Water Deprivation Findings in a Genetically Confirmed Case of Nephrogenic DI

Time	Weight (kg)	Urine output	Urine osmolality	Serum osmolality	Serum sodium	Symptoms	AVP
7 am	75.4		57	311	141		
8 am	75.10					Thirst	
9 am	74.15	1000	58	308	143	Thirst	
10 am	72.85	1000				Thirst	
11 am	72.45	800	64	320	146	Thirst	44.1 pg/mL
AVP 5 U SC (oral liquids allowed)							
12 pm			80			Thirst	
1 pm		800	65		141	Thirst	
2 pm			50			Thirst	
3 pm	76.25	700	54		140	Thirst	

In the above patient, due to subnormal rise in urine osmolality (25%) following administration of 5 U AVP which is less than the 50% cutoff, the patient was diagnosed to have diabetes insipidus due to a non-pituitary cause (nephrogenic DI).

1.14 Patient Instructions Sheet

1.14.1 Instructions to the Patient for Dexamethasone Suppression Test

1. Buy dexamethasone 0.5 mg_____ tabs, and get it checked by your doctor.
2. Give your blood sample for cortisol at 0800 h (8 am) on _____/_____/_____ at _____(place).
3. Take tab dexamethasone orally after giving blood sample as follows:

Date	Time	Tab Dexamethasone Dose

4. After the completion of last dose, give blood sample for cortisol at 0800h (8 am) on _____/_____/_____ at _____.

5. In case you forget to take a scheduled dose or the dose gets delayed by more than 3 h, do not take any further doses and test should be abandoned.

1.15 Instructions to the Patient for Urine Collection for 24-h Urinary Free Cortisol Test

1. You will need the following things:
   - A plastic container to collect the urine
   - A clean bottle to store the urine (should be of at least 2 l capacity, preferably 5 l)
   - A refrigerator
   - A black marker for labelling
2. Procedure for 24-h urinary collection:
   - Choose a day to collect urine, preferably on a day when you are at home the entire day.
   - Write your name and CR number on the bottle with black marker.
   - On the day of collection, record the date and time of first passing urine after you wake up. This urine sample should be discarded. The recorded time is the beginning of the 24-h urine collection time. (For example, if you wake up at 7:00 am, then discard the first passed urine. From this time onwards till 7:00 am the next day, collect all urine.)
   - After this, all urine samples have to be collected into the plastic container and transferred to the bottle. Even the urine passed during defecation should be collected.
   - Exactly 24 h after the starting of collection time, pass urine one last time. Collect this into the storage bottle and note down the date and time of completion of 24-h urinary collection.
   - Store the bottle in a refrigerator until you are ready to drop it off.
   - Transport the urine sample to the lab within 48 h of the last urine collection.
3. Precautions while performing 24-h urine collection:
   - Do not drink excessive amount of fluid during urine collection.
   - Avoid using glucocorticoids in any form, including topical.
   - Urine should be stored in a bottle in refrigerator until the sample is sent to the lab.

1.16 Instructions to the Patient for 11 pm Awake Cortisol and ACTH

1. Take prior appointment from your doctor.
2. Bring the following materials along with you: two 5 mL syringe and two EDTA (purple top) vials.

3. Come to the designated place _____ _____ at 9 pm.
4. You can take your dinner at your usual time.
5. After arriving to the specified place, rest from 9 pm to 11 pm. Avoid any exertion or physical activity.
6. If you are taking any of the following drugs, Hisone, Wysolone, prednisolone and prednisone, these should be stopped at least 24 h prior to blood sampling.
7. The blood sample will be kept in the department of endocrinology, and you should collect it the next morning from the same place and deposit it at the designated place.

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Endocrinology Evaluation Protocol: Diabetes and Thyroid

2

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Contents

2.1 Standard Oral Glucose Tolerance Test (OGTT) 43
2.1.1 Aim 43
2.1.2 Indications [1] 44
2.1.3 Preparation 44
2.1.4 Prerequisites 44
2.1.5 Procedure 44
2.1.6 Interpretation (Table 2.1) 44
2.1.7 Troubleshooting 45
2.2 Gestational Diabetes Mellitus (GDM) 45
2.2.1 Procedure (One-Step Approach) 45
2.2.2 Two-Step Approach in the Diagnosis of GDM 45
2.3 Glucagon-Stimulated C-Peptide Test 46
2.3.1 Principle 46
2.3.2 Aim 46
2.3.3 Indication [7] 46
2.3.4 Contraindication 46
2.3.5 Preparation 46
2.3.6 Procedure 46
2.3.7 Interpretation [7] 47
2.3.8 Adverse Events 47
2.4 Mixed-Meal Test (MMT) 47
2.4.1 Aim 47
2.4.2 Indications 47
2.4.3 Contraindication 47
2.4.4 Preparation 47
2.4.5 Prerequisite 48
2.4.6 Procedure [8] 48
2.4.7 Interpretation [10] 49

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2.5 Prolonged Supervised Fast (72-h Fast) 49
2.5.1 Principle 49
2.5.2 Aim 49
2.5.3 Indication 49
2.5.4 Contraindication 49
2.5.5 Preparation 50
2.5.6 Prerequisite 50
2.5.7 Procedure 50
2.5.8 At the Termination of Test 51
2.5.9 Interpretation [10] 52
2.6 Selective Arterial Calcium Stimulation (SACS) Test 54
2.6.1 Aim 54
2.6.2 Indication 54
2.6.3 Contraindications 54
2.6.4 Prerequisite 54
2.6.5 Preparation 55
2.6.6 Procedure [11] 55
2.6.7 Interpretation [11] 55
2.6.8 Complications 57
2.7 Thyrotrophin-Releasing Hormone (TRH) Test 57
2.7.1 Aim 57
2.7.2 Indication 57
2.7.3 Contraindications 57
2.7.4 Preparation 58
2.7.5 Procedure 58
2.7.6 Interpretation [13] (Table 2.5) 58
2.7.7 Side Effects 58
2.8 T3 Suppression Test 59
2.8.1 Principle 59
2.8.2 Aim 59
2.8.3 Indication 59
2.8.4 Contraindications 59
2.8.5 Prerequisite 59
2.8.6 Preparation 59
2.8.7 Procedure 60
2.8.8 Sampling 60
2.8.9 Interpretation [15] 60
2.8.10 Interpretation 62
Suggested Reading 62

Abbreviations

2-h PG	2-hour plasma glucose
ADA	American Diabetes Association
BG	Blood glucose
BHB	Beta-hydroxybutyrate
CBG	Capillary blood glucose

CECT	Contrast-enhanced computed tomography
CEMRI	Contrast-enhanced MRI sella
CPK	Creatinine phosphokinase
DPA	Dorsal pancreatic artery
EDTA	Ethylenediaminetetraacetic acid
FPG	Fasting plasma glucose
FT3	Free tri-iodo thyronine
FT4	Free tetra-iodo thyronine
GDA	Gastroduodenal artery
GDM	Gestational diabetes mellitus
IGT	Impaired glucose tolerance
IM	Intramuscular
IV	Intravenous
MEN-I	Multiple endocrine neoplasia type I
mHVI	Maximum hepatic venous insulin concentration
MIP	Maximum intensity projection
MMT	Mixed meal tolerance test
MODY	Maturity-onset diabetes of young
NIPH	Non-insulinoma pancreatogenous hypoglycemia
OGTT	Oral glucose tolerance test
PCOD	Polycystic ovarian disease
PET-CT	Positron-emission tomography-computed tomography
PGBH	Post-gastric bypass hypoglycemia
PRL	Prolactin
rHVI	Relative hepatic venous insulin concentration
RTH	Resistance to thyroid hormone
SHBG	Sex hormone-binding globulin
SMA	Superior mesenteric artery
SPA	Splenic artery
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TRH	TSH-releasing hormone
TSH	Thyroid-stimulating hormone
TT3	Total tri-iodo thyronine
TT4	Total tetra-iodo thyronine

2.1 Standard Oral Glucose Tolerance Test (OGTT)

2.1.1 Aim

To determine the degree of dysglycemia.

2.1.2 Indications [1]

- To establish the diagnosis of diabetes mellitus
- To diagnose impaired glucose tolerance (IGT)
- Screening for gestational diabetes mellitus
- To diagnose diabetes in conditions with altered relationship between HbA1C and plasma glucose such as thalassemia

2.1.3 Preparation

- Ensure adequate dietary carbohydrate (150 g/day) for at least 3 days before the test [2].
- Overnight fast for 8–12 h (no caloric intake).

2.1.4 Prerequisites

- The patient should abstain from smoking or alcohol intake in the previous 24 h.

2.1.5 Procedure

- Insert cannula and draw baseline sample for blood glucose at time zero.
- Oral glucose load (75 g anhydrous glucose or 82.5 g of glucose, which is readily available in the market) to be mixed in 300 mL of water and should be taken orally over 10 min.
- Repeat blood samples at 120 min after glucose intake (time should be calculated from the start of glucose intake).
- The patient should preferably be seated during the test.
- Always take a sample in an oxalate/fluoride vial for glucose estimation.

2.1.6 Interpretation (Table 2.1)

Table 2.1 Diagnosis of diabetes based on ADA and WHO criteria (Table 2.1)

	American Diabetes Association [3]		World Health Organization [4]	
Parameter	Diabetes	Prediabetes	Diabetes	Prediabetes
FPG	≥126 mg/dL	100–125 mg/dL (IFG)	≥126 mg/dL	110–125 mg/dL (IFG)
2-h PG (75 gm OGTT)	≥200 mg/dL	140–199 mg/dL (IGT)	≥200 mg/dL	140–199 mg/dL (IGT)

2.1.7 Troubleshooting

Some patients may develop nausea and occasionally vomiting. In such cases, the test should be abandoned and performed on another day.

2.2 Gestational Diabetes Mellitus (GDM)

- Perform a 75 g OGTT, with plasma glucose measurement when the patient is fasting and at 1 and 2 h.
- The test should be performed at 24–28 weeks of gestation in women not previously diagnosed with diabetes.
- However, fasting plasma glucose between 92 mg/dL and 125 mg/dL before 24 weeks of gestation is defined as *early GDM* as per the IADPSG [5].

2.2.1 Procedure (One-Step Approach)

- The OGTT should be performed in the morning after an overnight fast of at least 8 h.
- Insert cannula and draw baseline sample for blood glucose at time zero.
- Oral glucose load (75 g anhydrous glucose or 82.5 g of glucose, which is readily available in the market) to be mixed in 300 mL water and should be taken orally over 10 min.
- Repeat samples for blood glucose at 60 and 120 min after the glucose load.
- The patient should preferably be seated during the test.

The diagnosis of GDM made when any one of the following criteria are met (IADPSG criteria) [6]:

- Fasting: ≥92 mg/dL (5.1 mmol/L)
- 1 h: ≥180 mg/dL (10.0 mmol/L)
- 2 h: ≥153 mg/dL (8.5 mmol/L)

2.2.2 Two-Step Approach in the Diagnosis of GDM

- *Step 1*: Perform a 50 g glucose load (non-fasting) test and measure plasma glucose at 1 h between 24 and 28 weeks of gestation in women not previously diagnosed with diabetes.

  If plasma glucose at 1 h after a glucose load is ≥130 mg/dL, proceed to a 100 g oral glucose challenge test.
- *Step 2:* 100 g OGTT should be performed in a fasting state, and diagnosis of GDM is made when at least two of the following four criteria are met (Carpenter-Coustan criteria).

- Fasting ≥95 mg/dL
- 1 h ≥180 mg/dL
- 2 h ≥155 mg/dL
- 3 h ≥140 mg/dL

*We follow a one-step strategy at our institute.
** Unit conversion for plasma glucose: 18 mg/dL = 1 mmol/L.

2.3 Glucagon-Stimulated C-Peptide Test

2.3.1 Principle

Glucagon induces glycogenolysis and increases plasma glucose, which consequently increases insulin and C-peptide release.

2.3.2 Aim

- To estimate residual beta-cell function in type 1 diabetes mellitus
- To test the ability to release stored hepatic glycogen, useful in the evaluation of suspected hepatic glycogen metabolic disorders

2.3.3 Indication [7]

- To differentiate T1DM from T2DM or MODY

2.3.4 Contraindication

- In intercurrent illness or in those who have not eaten for more than 48 h
- Uncontrolled hypertension
- Pheochromocytoma

2.3.5 Preparation

After 8–12-h fasting and with blood glucose >70 mg/dL, glucagon is administered at a dose of 0.03 mg/kg body weight (maximum dose 1 mg IV or IM).

2.3.6 Procedure

- Insert IV cannula.
- Take baseline sample for glucose, C-peptide, and insulin.

- Administer glucagon IM or dilute in 5 mL normal saline and give over 10 s intravenously.
- Take a sample for glucose, C-peptide, and insulin at 2, 4, and 6 min.

2.3.7 Interpretation [7]

- Glucagon-stimulated C-peptide less than 0.2 nmol/l (0.6 ng/mL) suggests T1DM.
- A normal response is a rise in plasma glucose levels. Absent response shows hepatic glycogen metabolism abnormalities.

2.3.8 Adverse Events

- Nausea and vomiting
- Rebound or persistent hypoglycemia, necessitating glucose administration

*Unit convertor for C-peptide: 1 nmol/L = 1000 pmol/L = 3 ng/mL.

2.4 Mixed-Meal Test (MMT)

2.4.1 Aim

To ascertain the etiology of post-meal hypoglycemia

2.4.2 Indications

- To document reactive postprandial hypoglycemia
- Quantification of beta-cell function
- Evaluation of post-gastric bypass hypoglycemia (PGBH)
- Suspicion of hyperinsulinemic hypoglycemia when the prolonged fast test is inconclusive

2.4.3 Contraindication

- Severe gastroparesis
- Persistent vomiting due to other systemic illnesses

2.4.4 Preparation

- Ensure adequate dietary carbohydrates (250 g/day) for at least 3 days before the test.

- 8–12-h overnight fast.
- In a case of suspected insulinoma who presented with postprandial hypoglycemia, MMT should be performed before the 72-h fast test.

2.4.5 Prerequisite

- The test should be performed under supervision in the morning after the overnight fast.
- Ensure patent IV line.
- Label vials for sample collection.
- Measures to treat hypoglycemia at the appropriate time.

2.4.6 Procedure [8]

- Insert cannula and take a baseline blood sample for glucose ($t = 0$).
- Give mixed meal* or food suspected of inducing hypoglycemia (if not feasible, then Ensure plus powder 6 Kcal/kg body weight dissolved in 300 mL plain water and ingested over 10 min).
- Observe the patient for symptoms and/or signs of hypoglycemia, and keep a written log of all symptoms, time from the start of meal ingestion (Table 2.2).
- Once hypoglycemia (PG <45 mg/dL or <55 mg/dL if symptomatic) is confirmed from the laboratory report, collect samples for plasma insulin, C-peptide, and proinsulin and send them for processing.
- Mixed-meal tests should be interpreted based on laboratory-measured plasma glucose concentration, not those estimated with a point-of-care glucometer.
- If it is necessary to treat hypoglycemia before 300 min because of severe symptoms, obtain samples before administrating glucose.

Table 2.2 Table depicting the checklist for sampling parameters during the mixed-meal test

Sampling time (min)	Symptoms of hypoglycemia	Vitals (BP and pulse)	CBG	PG (lab)	Sample for C-peptide, and insulin at the time of symptomatic hypoglycemia
30 min					
60 min					
90 min					
120 min					
150 min					
180 min					
210 min					
240 min					
270 min					
300 min					

BP blood pressure, *CBG* capillary blood glucose, *PG* plasma glucose

***Suggested mixed meal (alternative to Ensure powder) [9]:**
- 30 g Cornflakes
- 250 mL Semi-skimmed milk
- 4.5 g Sachet sugar
- 170 mL Unsweetened orange juice
- 40 g (Medium) slice white bread
- 10 g Portion butter
- 50 g Piece cheddar cheese
- 100 g Low-fat fruit yogurt

Ensure powder contains (per 100 g):
- 14.20 g Protein/56.8 kcal/13.85%
- 10.5 g Fat/94.5 kcal/23.05%
- 62.98 g CHO/251.9 kcal/61.44%

2.4.7 Interpretation [10]

- As per the flow diagram (mentioned in 72-h fasting test)

2.5 Prolonged Supervised Fast (72-h Fast)

2.5.1 Principle

- Prolonged fasting induces hypoglycemia in subjects with endogenous hyperinsulinemia, but they will not develop ketosis while normal subjects develop ketosis even in the absence of hypoglycemia.

2.5.2 Aim

To establish a diagnosis in cases of unexplained spontaneous hypoglycemia

2.5.3 Indication

- Seemingly healthy individual with unexplained hypoglycemia

2.5.4 Contraindication

- Pregnancy
- Critical illness

2.5.5 Preparation

- Patient should be hospitalized, and the test should be performed under close supervision.
- Measures to treat hypoglycemia (glucose oral and/or iv) should be available bedside.
- Ensure that protocol is well understood by the patient and nursing staff.
- Contact lab to ensure availability of glucose results urgently.

2.5.6 Prerequisite

- Documentation of Whipple's triad (low blood glucose with symptoms of hypoglycemia, which resolve with glucose supplementation) is the cornerstone.
- Discontinue diazoxide, octreotide, glucocorticoids, or other drugs used to treat hypoglycemia for a week before the initiation of the 72-h fast.

2.5.7 Procedure

- Ensure patent IV line.
- The test should be performed in the ward under close supervision.
- The test starts from early morning if the patient has a history of recurrent spontaneous hypoglycemia.
- Only water and noncaloric beverages may be allowed.
- The patient should be active during the waking hours.
- Measure blood glucose every 4 h initially. Once blood glucose falls below 70 mg/dL, frequency should be increased to at least one hourly and whenever symptomatic. More frequent monitoring is advisable at night when the patient is asleep.
- The test should continue to the point till hypoglycemic symptoms are documented with plasma glucose of less than 55 mg/dL. If the patient does not have symptoms, then the fast can be terminated when:
  - RBG <45 mg/dL
  - RBG <55 mg/dL and previously documented Whipple's triad
  - 72 h have elapsed without symptoms
- Progressive rise in beta-hydroxybutyrate level (BHB) (≥2.7 mmol/l) during fasting rules out endogenous insulin excess as the cause for hypoglycemia (Table 2.3).

Table 2.3 Table depicting the checklist for sampling parameters during the prolonged supervised fast test

Day	Symptoms of hypoglycemia	Vitals (BP, pulse)	CBG	PG (from lab) to confirm hypoglycemia	Serum ketone	Sample for insulin, and C-peptide at the time of symptomatic hypoglycemia
1			Every 4 h		8 hourly	
2			Every 2 h		6 hourly	
3			Every 1 h		2 hourly	

- Collect a sample for insulin and C-peptide and send it for processing after documenting hypoglycemia from the lab (PG <55 mg/dL from the lab).
- Sampling frequency for capillary blood glucose (CBG) depends on symptoms of hypoglycemia and may vary from person to person, subject to individual variability.

2.5.8 At the Termination of Test

- Send glucose for urgent analysis. If lab glucose also confirms hypoglycemia, then send the corresponding sample for processing of insulin and C-peptide.
- If hypoglycemia was not evident after a 72-h fast, intravenous glucagon test can be done to assess the presence or absence of liver glycogen. Glucagon is given at a dose of 1 mg iv, and blood glucose is monitored at baseline and 10, 20, and 30 min postinjection. A rise in blood glucose by more than 25 mg/dL indicates the presence of residual liver glycogen, an indirect evidence for insulin (or insulin-like factor) secretion.
- Sample for circulating hypoglycemic agents and antibodies to insulin, ordered in clinically indicated individuals if the facilities are available.

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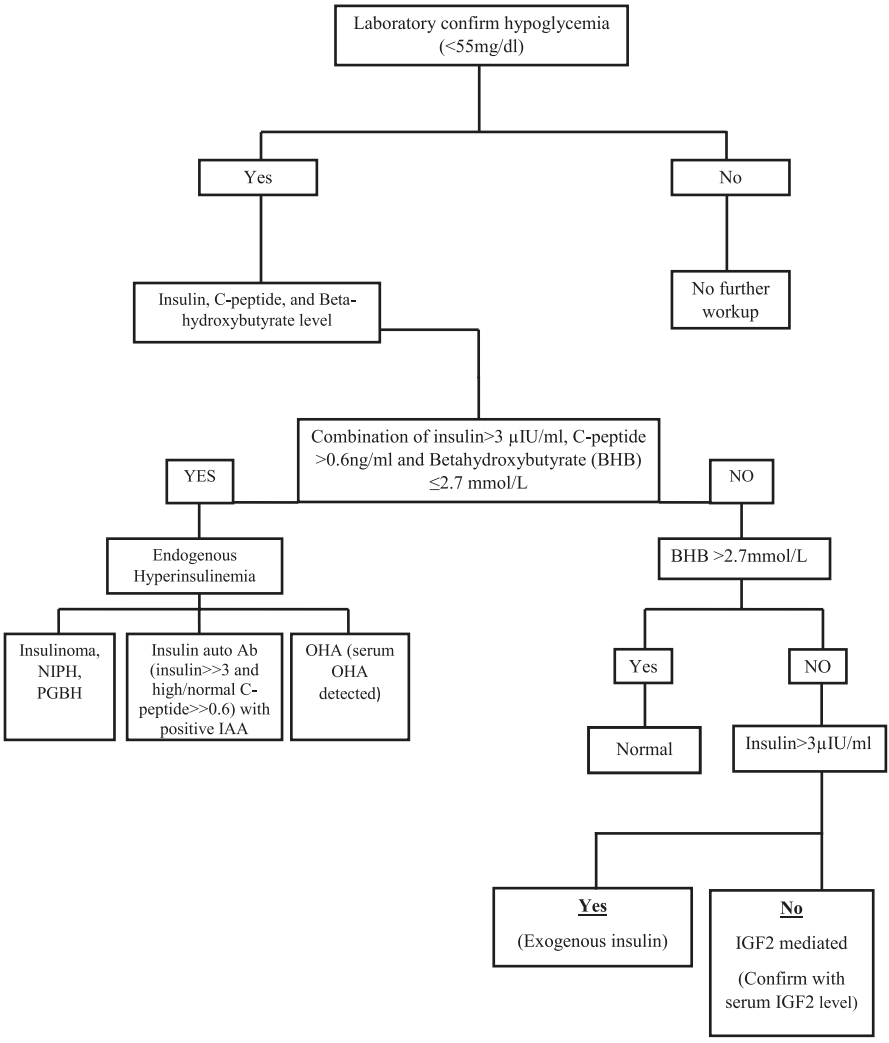
2.5.9 Interpretation [10]



Abbreviation: *NIPH* Non-insulinoma pancreatogenous hypoglycemia, *PGBH* Post-gastric bypass hypoglycemia, *OHA* Oral hypoglycemic agent

*Unit conversion for insulin: 1 pmol/L = 0.144 µIU/mL.

*Unit conversion for beta-hydroxybutyrate: 1 mmol/L = 10.41 mg/dL.

*Unit convertor for C-peptide: 1 nmol/L = 1000 pmol/L = 3 ng/mL.

Case 7: Prolonged Supervised 72-h Fast for Endogenous Hyperinsulinemic Hypoglycemia

A 55-year-old female presented with multiple episodes of fasting hypoglycemia for the past 3 months. She also complained of weight gain, which was unintentional

and, on examination, she had grade III acanthosis nigricans. She was suspected to have endogenous hyperinsulinemic hypoglycemia, which required confirmation by the 72-h fast. The prolonged supervised fast revealed hypoglycemia (random plasma glucose 40 mg/dL) at 14 h. Concurrent insulin was 12.5 uIU/ml, and C-peptide was 3.6 ng/mL. Whole-body Ga-68 exendin PET-CT was done, which showed an abnormal focus of tracer uptake in the right paramedian location in the abdomen, which on transaxial CECT and fused PET-CT images localized to an intensely tracer avid heterogeneously enhancing ill-defined lesion (~1.4 × 1.8 cm, SUVmax 27.0) in the uncinate process of the pancreas (black arrows in Fig. 2.1).

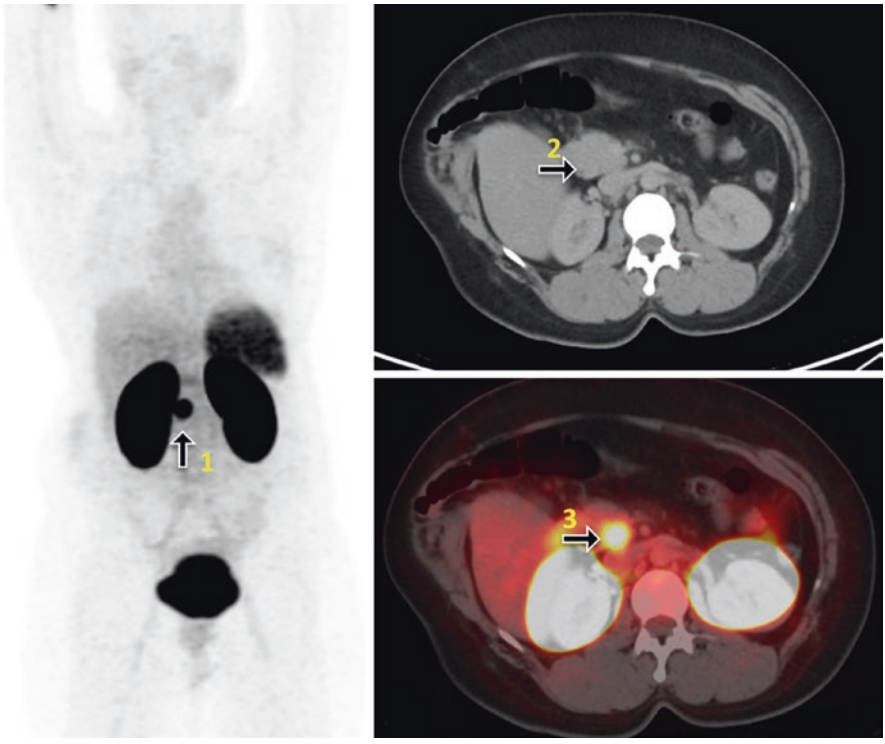


Fig. 2.1 Panel of photographs of a 55-year-old female who presented with fasting hypoglycemia. (1) Whole-body maximum intensity projection (MIP) image (A) of Ga-68 exendin PET-CT depicting abnormal focus of tracer (Ga-68 exendin) uptake in the right paramedian location corresponding to the source of endogenous hyperinsulinemia. (2) Mass in the uncinate process of pancreas on CECT. (3) Intensely avid heterogeneous ill-defined lesion in the uncinate process of the pancreas

2.6 Selective Arterial Calcium Stimulation (SACS) Test

2.6.1 Aim

To localize the source of excessive insulin production

2.6.1.1 Principle

Calcium is a known stimulator of insulin secretion. Exogenous intra-arterial calcium in the large arteries is sequentially injected with weight-based dosage of calcium gluconate. This differentially stimulates insulin secretion from the culprit or dominant pathologic lesion.

2.6.2 Indication

Localization of insulinoma in the following conditions:

- Patients with endogenous hyperinsulinemia and negative noninvasive imaging testing.
- MEN1 patients with multiple pancreatic lesions on imaging, to decide which lesion is functional.
- Patients with postprandial hypoglycemia where the functionality of any lesion identified on cross-sectional imaging needs to be confirmed (when functional imaging, e.g., 68-Ga exendin 4 PET-CT, is non-confirmatory or having multiple lesions).
- Patients with prior failed pancreatic exploration need to confirm that lesion(s) observed on cross-sectional imaging are functional.

2.6.3 Contraindications

- Allergy to contrast dye
- Ischemic heart disease
- Advanced heart failure—orthopnea
- Severe bleeding tendency (if the patient is on aspirin/clopidogrel, discuss with a radiologist)

2.6.4 Prerequisite

- Biochemically proven endogenous hyperinsulinemia.
- Stop diazoxide 7 days before the procedure [elimination half-life variable 21–36 h].

2.6.5 Preparation

- Take written, informed consent.
- Before the test, patient should fast for at least 4 h. Have 5% dextrose drips available; if hypoglycemic, administer at a rate to maintain blood glucose at about 54–90 mg/dL (not more).
- Keep EDTA vials ready with proper labeling to collect the sample (Table 2.4).
- Prepare study sheet with an order of arterial injection (artery supplying lesion most likely based on imaging studies to cannulate at last).

2.6.6 Procedure [11]

- Under fluoroscopic guidance, the venous catheter is positioned in the right hepatic vein for blood sampling.
- Perform standard pancreatic arteriography for selective catheterization of celiac, gastroduodenal (GDA), splenic artery (SPA), and superior mesenteric artery (SMA) as well as dorsal pancreatic artery (DPA) if necessary (Fig. 2.2).
- Following diagnostic arteriography, inject (as rapid bolus) calcium gluconate in a dose of 0.025 mmol per kg (10 mL of 10% calcium gluconate consists of 2.23 mmol) diluted in 5 mL of saline sequentially into GDA, SPA (distal followed by proximal), SMA, and DPA. Allow at least 5 min between each arterial stimulation.
- Separate the plasma for insulin estimation within 30 min, and store in ice if the procedure is prolonged.

2.6.7 Interpretation [11]

- A word of caution regarding the anatomical variations and considerable territorial overlap in the arterial supply of the pancreas is necessary. Discussion with the interventional radiologist regarding the same is of paramount importance. In the presence of DPA replaced by SMA, the SMA may perfuse the entire pancreas.

Table 2.4 Table depicting the checklist for sampling parameters during the selective arterial calcium stimulation test

Catheterization of the artery for bolus calcium gluconate injection (artery that is most likely supplying lesion to be cannulated last)	Catheterization of right hepatic vein for basal blood sampling (insulin) at		Sampling from a right hepatic vein after iv calcium gluconate stimulation (for insulin) at		
	−120 s	0 s	20 s	40 s	60 s
Gastroduodenal artery					
Proximal splenic artery					
Superior mesenteric artery					
Dorsal pancreatic artery (DPA)					

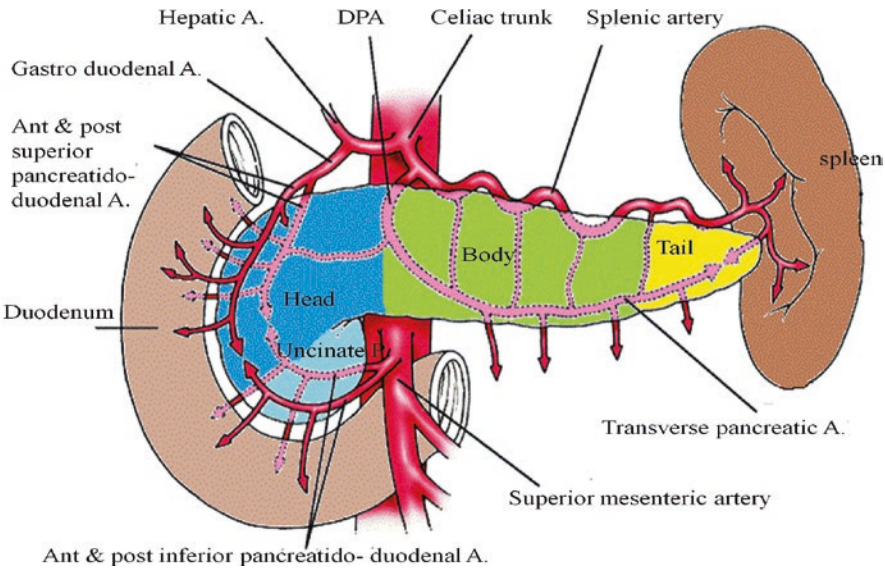


Fig. 2.2 Schematic representation of the arterial supply of the pancreas. The gastroduodenal artery supplies the superior pancreatic head and neck, the superior mesenteric artery supplies the inferior pancreatic head and uncinate process, and the splenic artery supplies the pancreatic body and tail

In the presence of celiac stenosis, calcium injection into the GDA does not provide localizing information. In such cases, the SMA may supply the entire pancreas via retrograde flow through the GDA and common hepatic artery further complicating localization.

- The pancreatic artery demonstrating the highest relative fold increase in hepatic venous insulin concentration over baseline following calcium injection is labeled as the dominant artery.
- The maximum hepatic venous insulin concentration (mHVI) is the highest absolute hepatic venous insulin concentration after calcium injection.
- The relative fold increase in hepatic venous insulin concentration (rHVI) is the fold increase in hepatic venous insulin concentration over baseline following calcium injection in the dominant artery.
- The mHVI of >83.1 μIU/mL has sensitivity and specificity of 83.3% and 93.2%, respectively, for insulinoma. While rHVI of >7.75 μIU/mL has sensitivity and specificity of 81% and 77%, respectively, for diagnosing insulinoma.
- In a Mayo Clinic series of 116 patients with endogenous hyperinsulinemia and inconclusive noninvasive imaging who underwent SACST, 73.8% of patients with insulinoma showed biochemical localization to a single arterial territory, while 73% of patients with nesidioblastosis had localization in two or more arterial territories. mHVI cutoff values of 91.5 IU/mL and 263.5 IU/mL were 95% and 100% specific for insulinoma, respectively. A 19-fold increase in rHVI over baseline was 99% specific for insulinoma.[11]

- The criterion for localization is a twofold rise in insulin in the 20-, 40-, or 60-s hepatic vein samples following injection into the relevant arterial territory.
- It is advisable to stimulate both the distal and proximal part of splenic artery. If insulin spike is noted after calcium injection in the distal SPA but not with proximal SPA, the lesion is likely in the tail of the pancreas, whereas if the lesion is in the body of the pancreas, there will be a response with calcium injection in both the proximal and distal splenic artery.

2.6.8 Complications

- Flushing and nausea may follow calcium injection.
- Risks of angiography itself: bleeding from sheath sites, thrombosis/dissection of the femoral artery and visceral arteries, and dye allergy.
- *Unit conversion for insulin: 1 pmol/L = 0.144 μIU/mL.
- *Unit convertor for C-peptide: 1 nmol/L = 1000 pmol/L = 3 ng/mL.
- *Unit convertor for calcium gluconate: 1 g salt =4.65 mEq =93 mg = 2.32 mmol elemental Ca.

2.7 Thyrotrophin-Releasing Hormone (TRH) Test

2.7.1 Aim

To stimulate hypothalamo-pituitary-thyroid axis.

2.7.1.1 Principle

TRH, by binding to the TRH receptors on the thyrotropes, stimulates them to release TSH in normal individuals. This excursion in the level of TSH is higher in cases of RTH than with TSHoma [12].

2.7.2 Indication

- To differentiate TSHoma from resistant thyroid hormone (RTH) syndrome
- To diagnose primary hypothalamic disorder (TRH deficiency)

2.7.3 Contraindications

- Severe hypothyroidism on levothyroxine supplement

2.7.4 Preparation

- Levothyroxine should be stopped for at least 3 weeks prior to the test.
- Overnight fasting is not necessary.
- Ensure patent IV line.
- TRH is to be administered intravenously at a dose of 200 mcg in adults and 5–7 mcg/kg/BW in children (Protirelin® 200 mcg in 0.2 mL).
- Pre-labelled EDTA (purple-cap) vials for sample collection.

2.7.5 Procedure

- Secure intravenous cannula.
- Take baseline blood sample for TSH and thyroxine (T4).
- Inject TRH slowly IV over 2 min.
- Flush cannula with heparin/saline.
- A sampling at baseline (zero), 20 min and 60 min.

2.7.6 Interpretation [13] (Table 2.5)

2.7.7 Side Effects

- Transient nausea, sensation of warmth, flushing, metallic taste, and/or urge to micturate, typically lasting less than 1 min, are frequently experienced by patients.
- Transient hypertension or hypotension may occur in some cases.

*Unit convertor for TSH: 1 μIU/mL = 1 mIU/L.
*Unit convertor for T4: 1 nmol/L = 0.0777 μg/dL.

Table 2.5 Table depicting the interpretation of the thyrotropin stimulation test (Table 2.5)

Time	Sample for		
Basal	TSH, T4	Result	Interpretation
20 min	TSH	TSH increment to 5–20 μg/dL	Normal response
		TSH increment <1.5-fold with basal elevated T4	TSHoma
		Exaggerated TSH (>fivefold) response with elevated basal T4	RTH
60 min	TSH	60 min TSH >20 min TSH value with low basal T4	Primary hypothalamic disorder

2.8 T3 Suppression Test

2.8.1 Principle

In RTH, negative feedback of hypothalamic pituitary thyroid axis is set at a higher threshold. Hence, partial suppression of TSH is seen at supraphysiological dose of T3 administration, while no response is seen in TSHoma due to T3 resistance [14].

2.8.2 Aim

To suppress the hypothalamo-pituitary-thyroid (HPT) axis

2.8.3 Indication

- To differentiate RTH from TSHoma

2.8.4 Contraindications

- Coronary artery disease
- Heart failure

2.8.5 Prerequisite

Confirm hyperthyroidism with non-suppressed TSH (secondary hyperthyroidism) by documenting elevated free thyroid hormone levels.

2.8.6 Preparation

- Admit the patient to the hospital 1 day before testing.
- Secure a patent IV line.
- The subject should be on a diet constant in calories and protein, tailored to the individual's home-based diet.
- Record dietary intake each day and its content in terms of carbohydrates, fats, and proteins.

2.8.7 Procedure

- The parameters to assess central and peripheral tissue effect of thyroid hormone in the basal state and during the administration of three incremental doses of T3 (liothyronine).
- The doses of LT3 as follows:
  - Days 1–3, 25 mcg BD 12 h apart
  - Days 4–6, 50 mcg BD 12 h apart
  - Days 7–9, 100 mcg bd 12 h apart
- Record BMR (TANITA) each day as soon as the patient wakes up.
- Record body weight in the morning every day and heart rate during sleep (between 0200 and 0400 h).

2.8.8 Sampling

- Testing for TSH, PRL, TT4, TT3, FT4, FT3, cholesterol, triglycerides, creatinine phosphokinase (CPK), ferritin, and SHBG should be performed in the basal sample before TRH administration. SHBG and TSH are measured on days 4, 7, and 10.

2.8.9 Interpretation [15]

- The combination of elevated thyroid hormones, suppressible TSH on T3 administration, and a lack of increment in SHBG is suggestive of RTH.
- Partial inhibition of TSH secretion after supraphysiological T3 suppression test (with doses of 100 or 200 mcg) may be seen in RTH.
- The lack of further increment in SHBG and suppression of TSH with T3 administration indicate TSHoma.

*Unit convertor for TSH: 1μIU/mL = 1 mIU/L.
*Unit convertor for T4: 1 nmol/L = 0.0777 μg/dL.
*Unit convertor for T3: 1 nmol/L = 0.651 ng/mL.

Case 8: Patient with Resistance to Thyroid Hormone

A 13-year-old boy presented with complaints of generalized weakness, lethargy, and poor weight gain since the age of 6 years. He also had a history of poor performance in school with difficulty in memorizing. There was no history of palpitations, increased sweating, hyperactivity, or rapid height gain. He however had a grade 1 goiter. Patient was born out of a non-consanguineous marriage, at full term with a birth weight of 2.5 kg. Patient subsequently achieved developmental milestones at appropriate age. He underwent thyroid function tests at the age of 6 years, which

revealed raised TT3 (3.03 ng/mL; normal = 0.6–2.0) and raised TT4 (23.70 μg/dL; normal = 4.5–12) with a normal TSH (4.05 μIU/mL; normal = 0.3–5.5). His FT3 and FT4 levels were also raised as shown in the table below. ^{99m}Tc pertechnetate scan was suggestive of increased trapping function (R > L) of both the lobes (uptake: right 1.8%, left 1.5%, total 3.2%). He was started on carbimazole 20 mg/day, which he continued taking for the next 7 years at a dose varying from 10 to 20 mg/day. There was no relief in symptoms or worsening with the treatment. 8 years back, the patient's father got tested in view of generalized weakness and was found to have the same pattern of thyroid function test, i.e., raised T3 and T4 and normal TSH, and was subsequently prescribed carbimazole 15 mg/day, which he continued to take intermittently. His FT3 and FT4 were also found to be raised. Patient also had a cousin (paternal aunt's daughter) who had come to endocrine OPD 6 months back with a complaint of goiter and on investigations found to have a similar pattern of thyroid tests.

2.8.9.1 Patient's TFT

FT3	7.65 pg/mL (2.39–6.79)
FT4	2.15 ng/dL (0.58–1.64)
TSH	2.44 μIU/mL (0.34–5.6)

2.8.9.2 Father's TFT

FT3	7.34 pg/mL (2.39–6.79)
FT4	2.29 ng/dL (0.58–1.64)
TSH	1.73 μIU/mL (0.34–5.6)

CEMRI sella was normal. Next, T3 suppression test was conducted to differentiate resistance to thyroid hormone (RTH) and thyrotropinoma.

	Basal (1/6/19)	5/6/19	8/6/19	11/6/19
T3 (ng/mL)	2.3	3.86	4.56	5.99
T4 (mcg/dL)	11	14.27	10.90	9.88
TSH (mIU/mL)	3.52	2.03	1.09	0.261
Prolactin (ng/mL)	17.83	7.27	9.54	
Cholesterol (mg/dL)	247	143	154	157
Triglycerides(mg/dL)	261	247	245	318.8
CPK (U/L)	97	111	134	97
Ferritin (mcg/L)	68.3	26.2	21.01	24.47
ALP (IU/L)	348	286	283	285
Mg (mg/dL)	2.21	3.6	1.90	1.87
Heart rate (night) (/min)	53	58	62	60
Weight (kilograms)	44.5	43.5	45	44

A L G r a w a n y

2.8.10 Interpretation

Administration of liothyronine led to suppression of TSH in a dose-dependent manner, which favors diagnosis of RTH over thyrotropinoma. A concomitant decrease in T4 levels was also seen reflective of decreased TSH drive for T4 production. Other parameters mentioned in the table above are reflective of impaired peripheral tissue responses seen in cases of generalized RTH. Cholesterol and triglycerides were indicators of effect on lipid metabolism; administration of T3 did not decrease triglycerides indicating a state of impaired response to thyroid hormone. No change in CPK reflects impaired effect on protein catabolism. Failure of ferritin to rise was indicative of resistance of thyroid hormone action at liver. SHGB was not done because of nonavailability of assay. Similarly, there was no rise in heart rate, which is expected to occur with T3 administration.

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Endocrinology Evaluation Protocol: Adrenals

3

Kanhaiya Agrawal, Soham Mukherjee, and Rama Walia

Contents

3.1 Adrenal Insufficiency..... 64
3.1.1 Types of Test Available: Short Synacthen Test, Insulin Tolerance Test..... 64
3.1.2 Tests for Primary Hyperaldosteronism..... 68
3.1.3 Confirmatory Tests for Primary Aldosteronism..... 69
3.1.4 Tests to Differentiate Pseudo-Cushing's from Cushing's Syndrome..... 79
3.1.5 Test for ACTH-Independent Macronodular Adrenal Hyperplasia (AIMAH)..... 84
3.1.6 Mixed-Meal Stimulation Test (Details Have Been Mentioned Earlier in the Chapter)..... 85
Suggested Reading..... 90

Abbreviations

ACTH	Adrenocorticotropic hormone
ADH	Antidiuretic hormone
AI	Adrenal insufficiency
AIMAH	ACTH-independent macronodular adrenal hyperplasia
AVS	Adrenal venous sampling
CAH	Congenital adrenal hyperplasia
cAMP	Cyclic adenosine monophosphate
CBG	Cortisol-binding globulin
CKD	Chronic kidney disease
COC	Combined oral contraceptive pill
CSI	Contralateral suppression index

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DAST	Dexamethasone androgen suppression test
DHEAS	Dehydroepiandrosterone sulfate
ECG	Electrocardiogram
eGFR	Estimated glomerular filtration rate
GIP	Gastric inhibitory polypeptide
GnRH	Gonadotropin-releasing hormone
HRT	Hormone replacement therapy
IU	International units
IVC	Inferior vena cava
LDDST	Low-dose dexamethasone suppression test
LH-R	Luteinizing hormone receptor
OCP	Oral contraceptive pill
PAC	Plasma aldosterone concentration
PAI	Primary adrenal insufficiency
PCOS	Polycystic ovarian syndrome
PR	Pulse rate
PRA	Plasma renin activity
SERM	Selective estrogen receptor modulator
SST	Short synacthen test
VIP	Vasoactive intestinal peptide

3.1 Adrenal Insufficiency

3.1.1 Types of Test Available: Short Synacthen Test, Insulin Tolerance Test

3.1.1.1 Cosyntropin/Short Synacthen Test (SST)

Principle

Adrenal gland is maximally or supra-physiologically stimulated with exogenous synthetic ACTH to secrete cortisol and other ACTH-dependent adrenal hormones. The response to synacthen is subnormal in the primary adrenal insufficiency (due to destruction of adrenal gland) and secondary adrenal insufficiency (due to adrenocortical atrophy).

Pharmacodynamics and Pharmacokinetics

Synacthen is synthetic ACTH or tetracosactide (1–24 amino acids), which contains 250 mcg/mL; it starts to act within 5 min of administration. The maximum effect is achieved at 60 min by either IM or IV administration. It has an eliminiation half-life of 7 min. It is excreted in urine and does not require dose adjustment in conditions with renal or hepatic impairment.

Fig. 3.1 Photographs of a 20-year-old girl who presented with recurrent vomiting and weight loss, depicting the hyperpigmentation of the skin (**a**) and oral mucosa (**b**). Synacthen stimulation test showed non-stimulable cortisol (368 nmol/l), confirming primary adrenal insufficiency

Aim
To confirm the diagnosis of adrenal insufficiency (Fig. 3.1).

Indications
Primary adrenal insufficiency (PAI), secondary AI, congenital adrenal hyperplasia (CAH), recovery of hypothalamo-pituitary-adrenal (HPA) axis, and adrenal venous sampling (AVS).

Contraindications
The test is contraindicated in patients with known hypersensitivity to synacthen.

Preparation
The test can be performed on any time of the day, and fasting is not required.

Prerequisite
Withhold steroid intake in any form, at least 24 h (for hydrocortisone) and 72 h (for prednisone/prednisolone/dexamethasone) prior to testing.

SST may not be appropriate in case of recent pituitary insult either by surgery or by apoplexy-induced secondary adrenal insufficiency.

Procedure
A good-quality intravenous cannula is inserted, and patency is ensured before performing the test.

Baseline (0 min) blood samples are taken for ACTH (collected in pre-chilled EDTA vial) and cortisol in case of adrenal insufficiency and 17-hydroxyprogesterone/pregnenolone or 11-deoxycortisol based on the type of CAH suspected.

Table 3.1 Weight-based doses for short synacthen test

Age	Synacthen dose (IM or IV)
<1 year	15 mcg/kg (maximum is 125 mcg)
1–2 years	125 mcg
>2 years and adults	250 mcg

Table 3.2 Interpretation of values obtained during the synacthen stimulation test for diagnosis of the condition

Condition	Test performed	Values	Interpretation
Adrenal function	Serum cortisol (nmol/L)	<550	Deficient
		>550[a]	Sufficient
CAH (21-α-hydroxylase)	17-OH progesterone	<2 ng/mL	No CAH
		2–10 ng/mL	CAH other than 21-α-hydroxylase deficiency, NCCAH (heterozygous)
		>10 ng/mL	NCCAH
		>100 ng/mL	Classical CAH

[a]Above 430–450 nmol/L of serum cortisol suggestive of normal adrenal function by LC-MS

Synacthen is given either intravenously (over 2 min) or intramuscularly. The dose for various age groups has been described below (Table 3.1):

Thereafter, a blood sample for cortisol/other metabolites is withdrawn at 60 min postinjection (Table 3.2).

Pitfalls of SST

Falsely Stimulable ("Sufficient," i.e., Serum Cortisol >550 Nmol/L)
In patients with evolving adrenal insufficiency.
If performed in recent pituitary surgery/apoplexy.
If the patient has taken hydrocortisone within 24 h.
Conditions with increased CBG (patient on OCP or SERM or mitotane).

Falsely Non-stimulable ("Insufficient," i.e., Serum Cortisol <550 Nmol/L)
In critical illness.
Conditions with low albumin or CBG.

Adverse Events of Synacthen

Usually, it is not associated with any side effects.
Occasionally, hypersensitivity or skin rash can be seen.

3.1.1.2 Low-Dose Synacthen Stimulation Test (1 mcg)

Principle
Low-dose (1 mcg) synacthen is a sufficient stimulus for testing patients with secondary adrenal insufficiency.

Aim
To confirm secondary adrenal insufficiency.
It may also be used to evaluate evolving adrenal insufficiency.

Indications
- It has been advocated in secondary adrenal insufficiency.
- Not recommended in a critically ill patient.

Preparation
Diluting 250 mcg, i.e., 1 mL, of synacthen in 99 mL of 0.9% of normal saline.
0.1 mL of diluted solution contains 0.25 mcg.
Therefore, 0.4 mL contains 1 mcg of synacthen.
(16 units using 40 IU insulin syringe)

Shelf Life
Not studied.

Interpretation
Same as synacthen stimulation test.

Pitfalls
1mcg SST may be falsely low due to logistic issues (as synacthen peptides stick to the wall of the syringe).
This test is used off-label.

3.1.1.3 Acton Prolongatum Stimulation Test
It contains corticotrophin carboxymethylcellulose 60 IU/mL.
It has 1–39 amino acids.

Principle
Same as synacthen stimulation test.

Aim, Indications, and Contraindications
Same as synacthen stimulation test.

Procedure
25 IU of acton prolongatum is given intramuscularly, and a blood sample is drawn for cortisol at 60 min.

Dose Preparation
5 mL vial containing 300 IU; hence, 1 mL contains 60 IU. Need to give 25 IU, hence (5/300) * 25 = 0.4 mL, which corresponds to 0.4 × 40 (as insulin syringe is 40 IU/mL) = 16 units in a 40 IU (red) insulin syringe.

Interpretation
Same as synacthen stimulation test.

Unit Conversion

Test	Conventional unit (C)	SI unit
Serum cortisol	1 mcg/dL	27.59 nmol/L

3.1.2 Tests for Primary Hyperaldosteronism

3.1.2.1 Assessment of Plasma Aldosterone Concentration (PAC) and Plasma Renin Activity (PRA)

Aim
It is a screening test to diagnose the state of hyperaldosteronism.

Indications
- Sustained blood pressure >150/100 mm Hg.
- Hypertension resistance to three conventional antihypertensive drugs including diuretics.
- Blood pressure controlled on four or more antihypertensive drugs.
- Hypertension with spontaneous or diuretic-induced hypokalemia.
- Hypertension with adrenal incidentaloma.
- Hypertension with sleep apnea.
- Hypertension with a family history of early-onset hypertension or cerebrovascular accident (at a young age <40 years).

Contraindication
None.

Preparation/Prerequisite
Normokalemia.

Spironolactone should be stopped for at least 6 weeks prior to the test.

Procedure
Blood sampling can be accomplished by paired measurements of PAC and PRA/DRC (some laboratories provide DRC) in a random morning ambulatory blood sample, i.e., patient should walk for 2 h and then sit for 15 min, and then sampling

should be done (preferably obtained between 8 am and 10 am). The sample should be collected and transported at room temperature (no freezing).

Interpretation

PAC (ng/dL)/PRA (ng/mL/h) (ARR) of >10 (ng/dL)/(ng/mL/h) with suppressed PRA (<1.0 ng/mL/h) or DRC (<4 mU/L) should be subjected for confirmatory test.

In the setting of spontaneous hypokalemia and PRA <0.3 ng/mL/h (below detection limit) and PAC >20 ng/dL, there is no need for confirmatory test (Tables 3.3 and 3.4).

3.1.3 Confirmatory Tests for Primary Aldosteronism

3.1.3.1 Intravenous Saline Suppression Test

Principle

Normal saline provides a sodium load, resulting in suppression of the normal renin-angiotensin-aldosterone system. In patients with primary hyperaldosteronism, this sodium load fails to suppress the high and autonomous aldosterone secretion.

Aim

To confirm primary aldosteronism.

Indication

The patient who has a positive screening test for primary hyperaldosteronism (PAC/PRA) or strong clinical suspicion of hyperaldosteronism.

Table 3.3 Interpretation of values obtained during screening test for primary hyperaldosteronism

Test	Values (ng/dL)/(ng/mL/h)	Conditions
ARR (aldosterone/renin ratio, i.e., PAC/PRA)	4–10	Essential hypertension
	>10[a]	Suspicious of primary aldosteronism

[a] With PRA <0.6 ng/mL/h

Table 3.4 Medications that may affect the screening test for primary hyperaldosteronism

Medications	Effect on PAC	Effect on PRA	Effect on ARR
Diuretics	↑	↑↑	↓
Beta-blockers, alpha-2 agonist, NSAIDs	↓	↓↓	↑
ACE inhibitors, ARBs, CCBs (dihydropyridine)	↓	↑↑	↓

Contraindications
- Severe uncontrolled hypertension.
- Severe renal insufficiency.
- Heart failure.
- Cardiac arrhythmia.
- Severe hypokalemia.

Prerequisite
Normokalemia should be achieved with potassium supplements prior to the test.

Preparation
Patient to lie in recumbent position at 8 am for half an hour before starting the test.

Procedure
Intravenous 0.9% normal saline of 2 L is infused over 4 h (500ml/hr). Blood pressure, PR, and signs of fluid overload are monitored at frequent intervals. At the end of 4 h, a blood sample for plasma aldosterone concentration (PAC) is withdrawn. During the test, the patient is advised not to stand up or walk around, except for micturition (Table 3.5).

Troubleshooting
Patients undergoing this test may be at a higher risk of a further rise in blood pressure or developing fluid overload. So, cardiac and renal status needs to be assessed before the procedure. However, if fluid overload develops, then infusion should be immediately stopped and managed with diuretics.

3.1.3.2 Oral Sodium Loading Test

Aim
To confirm primary aldosteronism.

Principle
Principle of the test is the same as intravenous sodium loading test.

Table 3.5 Interpretation of values obtained during confirmatory testing for primary hyperaldosteronism

Plasma aldosterone concentration (PAC) ng/dL	Conditions
<5	Hyperaldosteronism ruled out, s/o essential hypertension or other causes
5–10	Indeterminate, retesting it later or another confirmatory test required
>10	Primary aldosteronism

Indication
A patient who has a positive screening test for hyperaldosteronism (PAC/PRA) or borderline response with strong clinical suspicion of hyperaldosteronism.

Contraindications
- Severe uncontrolled hypertension.
- Severe renal insufficiency (preferably avoided in eGFR <30 mL/min/m^2).

Heart Failure
- Cardiac arrhythmia.
- Severe hypokalemia.

Prerequisite
Normokalemia should be achieved with potassium supplements prior to the test. Potassium chloride supplementation may be needed before all confirmatory tests to maintain normokalemia. The target serum potassium value is 4 mmol/L, and sodium excretion rate is 3 mmol/kg body weight (min. 200 mmol per day).

Procedure
A high-sodium diet (>300 mEq/day or 18 gram/day in divided doses) for 3 days. Adherence to a high-salt diet is monitored by the measurement of urinary sodium concentration (Tables 3.6 and 3.7).

3.1.3.3 Fludrocortisone Suppression Test

Aim
To confirm primary aldosteronism.

Table 3.6 Interpretation of urinary aldosterone testing as confirmatory testing for primary hyperaldosteronism

Urinary aldosterone concentration (UAC) (mcg/24 h)	Conditions
<12	Normal response
>12	Primary aldosteronism with urinary Na + concentrations >200–250 mmol/day

Table 3.7 Table depicting the sensitivity and specificity of various stimulation tests used for confirming the diagnosis of primary hyperaldosteronism

Test	Cutoff	Sensitivity	Specificity
Intravenous saline suppression test	10 ng/dL	81%	96%
Oral sodium loading test	UAC >12 mcg/24 h	96%	93%
Fludrocortisone suppression test	6 ng/dL	93%	90%

A L G r a w a n y

Principle

The basic principle of the test is to orally administer fludrocortisone along with high-salt diet to ensure a high sodium load. This stimulates thirst and ADH, leading to fluid retention, hypervolemia, and consequent suppression of the RAAS in healthy subjects or those with essential hypertension. In primary hyperaldosteronism, the level of aldosterone is expected to remain high in the face of high sodium load.

Oral administration of fludrocortisone with high-salt diet
↓
Sodium retention
↓
Stimulation of thirst and ADH/aquaporin-2
↓
Water retention and hypervolemia
↓
Suppression of renin and endogenous aldosterone secretion (healthy/ Essential hypertension

However, the level will not decrease in patients with primary aldosteronism.

Indication

The patient who has a positive screening test for primary hyperaldosteronism (PAC/PRA) or borderline response with strong clinical suspicion of hyperaldosteronism.

Contraindication

- Severe uncontrolled hypertension.
- Severe renal insufficiency (preferably avoided in eGFR <30 mL/min/m^2).
- Heart failure.
- Cardiac arrhythmia.
- Severe hypokalemia (<2.5 mEq/L).

Prerequisites

To perform this test, patients usually require hospitalization. Potassium chloride supplementation may be needed before all confirmatory tests to maintain normokalemia. The target serum potassium value is 4 mmol/L, and sodium excretion rate is 3 mmol/kg body weight (min. 200 mmol per day).

Procedure

High-salt diet (10–12 g salt/day) and slow-release NaCl tablets 30 meq (2 g) TID along with tab fludrocortisone 100 mcg QID are prescribed for 4 days. Blood sample for cortisol estimation is done twice, at 0700 to 0800 h and again at 1000 h. Blood for PAC and PRA is withdrawn at 1000 h.

Cortisol is sampled between 7 and 8 AM to ensure a decline between early and late morning time points (so as to exclude false-positive high aldosterone values due to stress and corticotrophin influence) (Table 3.8).

3.1.3.4 Adrenal Venous Sampling

It is the gold standard to lateralize the source of aldosterone excess.

Aim

To lateralize the source of aldosterone excess.

Principle

Distinguishing unilateral and bilateral disease in primary hyperaldosteronism patients is critical to guide its management. It fills the gap of many limitations of imaging including the non-visualization of small tumors and the inability to detect the functionality of adenoma.

Indication

When surgical cure for hyperaldosteronism is being contemplated but there remains uncertainty about the source of the excess aldosterone (unilateral or bilateral) as in the following situations:

1. Primary aldosteronism with unilateral adenoma >1 cm in size; but patient is more than 35 years of age
2. Bilateral adrenal lesions on CT scan
   OR
   Unilateral mass lesion <1 cm in size
   OR
   Age >35 years with primary aldosteronism (unilateral or bilateral)

Bilateral disease (no masses in adrenal gland on either side) with nevertheless a high possibility of aldosterone-producing adenoma as evidenced by

(a) Serum aldosterone >30 ng/mL
(b) 24-h urinary aldosterone >30 mcg/day
(c) Spontaneous hypokalemia
(d) Moderate-to-severe hypertension
(e) Age <50 years

Table 3.8 Interpretation of values obtained during fludrocortisone suppression test for confirming the diagnosis of primary hyperaldosteronism

Plasma aldosterone concentration (PAC) ng/dL	Conditions
<6	Normal response
>6	Primary aldosteronism

3.1.3.5 Not Required/Indicated in the Following Cases

1. Younger patients aged <35 years having a unilateral adenoma >1 cm can proceed directly to surgery.
2. Patients with unilateral mass <1 cm size OR bilateral masses OR bilateral normal adrenals on CT scan with no features that increase the likelihood of a unilateral disease like aldosterone-producing adenoma (as mentioned above in points a) to e)). These patients may be treated medically.

Contraindication

- Patient refusing the procedure.
- Hypokalemia.
- Uncontrolled hypertension.

Preparation/Prerequisite

Written, informed consent explaining the pros and cons (invasive, expensive, failure, no conclusion, and challenging) of the procedure.

Normal potassium level.

The test should be preferably done in the morning, with the patient in the supine position for at least 1 h before sampling.

Spironolactone is to be avoided at least 4–6 weeks prior to the procedure (if spironolactone requirement is a must for blood pressure control, then document low plasma renin activity prior to the procedure performed).

Ideally, cortisol assay should be available on-site to assist in selective adrenal vein catheterization.

Avoidance of stress and anxiety to minimize the fluctuations of hormones is ideal.

Procedure

In our institute, stimulated sequential adrenal venous sampling is performed by intervention radiologists through the femoral approach.

Injection synacthen 50 mcg/h is administered intravenously starting 30 min prior to the procedure and given as continuous infusion till completion of the procedure.

When both the adrenal veins are catheterized (left followed by right), which were confirmed by fluoroscopy, blood sample for plasma aldosterone and cortisol is obtained from both the adrenal vein and inferior vena cava (IVC) below the renal vein (Table 3.9).

Interpretations

- *Selectivity index*
  - To determine whether the right or left adrenal is appropriately cannulated or not
  - It is based on the step-up gradient of adrenal hormone compared to that of the periphery.
  - We use cortisol; however, other studies have shown the use of 17-OHP or androstenedione as well.
  - Selectivity index— $\frac{\textit{Cortisol (adrenal vein right or left)}}{\textit{cortisol (IVC)}}$

Table 3.9 Parameters to be used during the adrenal venous sampling test to localize the source in case of primary hyperaldosteronism

	Cortisol	Aldosterone	Cortisol-corrected aldosterone	Cortisol-corrected aldosterone (dominant)/ cortisol-corrected aldosterone (nondominant) (lateralization index)	Cortisol-corrected aldosterone (nondominant)/ cortisol-corrected aldosterone (IVC) (contralateral suppression index)
Right AV					
Left AV					
Low IVC					

  - Cutoff of selectivity index:
    >5 suggests appropriate catheterization to the respective site
    <5 suggests inappropriate catheterization
- *Lateralization index:*
  - To determine the source of excess aldosterone excess.
  - Lateralization index = Cortisol-corrected aldosterone (dominant/higher functioning gland)/Cortisol-corrected aldosterone (nondominant).
  - *Cortisol-corrected aldosterone = Aldosterone ÷ cortisol
  - Cutoff of lateralization index:
    >4 suggests lateralized
    3–4 suggests gray zone; look for contralateral suppression index
    <3 suggests bilateral disease (hyperplasia)
- *Contralateral suppression index (CSI)*
  - To look for suppression of contralateral adrenal gland.
  - Assessed in case of the inappropriately catheterized adrenal gland.
  - When lateralization index falls in the gray zone (3–4).
  - Predict the good surgical outcome.
  - CSI = Cortisol-corrected aldosterone (nondominant)/Cortisol-corrected aldosterone (IVC)
  - *Cortisol-corrected aldosterone = Aldosterone÷cortisol
  - Cutoff CSI: ≤0.5 suggests contralateral disease.

Troubleshooting

In case of the sudden drop in blood pressure, suspect for adrenal hemorrhage or damage to large intra-abdominal vessels.

So, these patients should be managed with the intravenous rush of normal saline or injection hydrocortisone 100 mg (after withdrawing blood sample for cortisol).

Also, arrange the packed RBC (PRBC) or whole blood for transfusion.

Complications

Loin pain.

Adrenal hemorrhage <2% in experienced centers:

- Can occur up to 72 h of the procedure
- Usually related to contrast push

Contrast-induced acute kidney injury.

Case 10: Adrenal Venous Sampling for Primary Hyperaldosteronism

A 65-year-old gentleman presented with a history of hypertension for the past 25 years and recent episode of hypokalemic paralysis with potassium documented as 1.3 mEq/L. On workup, he was found to have PAC = 83.8 ng/dL, PRA <0.10 ng/mL/h, and ARR >838. Post-saline suppression, PAC was 47 ng/dL, which confirmed the diagnosis of primary hyperaldosteronism. MRI abdomen revealed (Fig. 3.2) an 18 mm × 15 mm sized adrenal nodule in the left adrenal gland.

- Normal right adrenal gland.
- Enlarged left adrenal gland with a nodule (18 x 15 mm Conn's adenoma).

Patient was then subjected to adrenal venous sampling (sequential) which showed the following results:

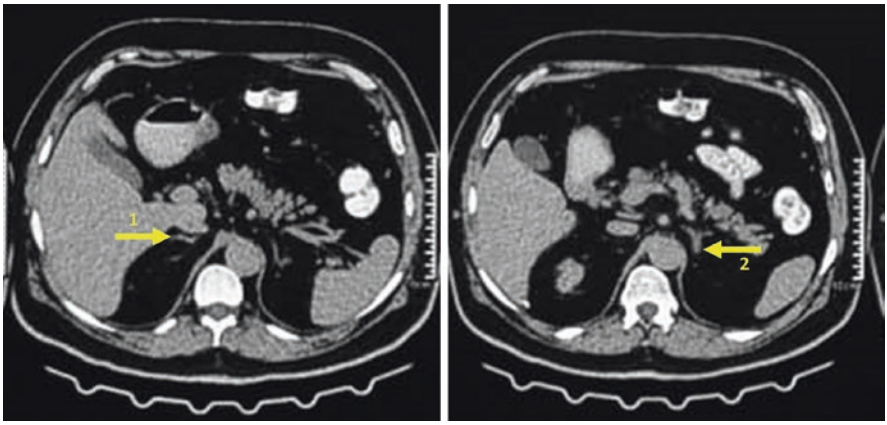

Fig. 3.2 Panel of photographs of the patient showing MRI of the adrenal glands with an appreciable nodule in the left adrenal

Site	Aldosterone (ng/dL)	Cortisol (nmol/L)	Aldosterone: cortisol (cortisol-corrected aldosterone)
Right	151.2	21,458	0.007
Left	2800	23,012	0.121
IVC	81.6	978	0.083

Selectivity index [cortisol (adrenal vein) / cortisol (IVC)]	
Right	21,458 / 978 = 21.94
Left	23,012 /978 = 23.52

Lateralization index (aldosterone:cortisol of dominant side / aldosterone:cortisol of nondominant side)	0.121/ 0.007 = 17.28
Suppression index (aldosterone:cortisol of nondominant side / aldosterone:cortisol of IVC)	0.007 / 0.083 = 0.08

The selectivity index was >5 on both the sides, which suggests appropriate catheterization. The lateralization index was >4, which suggested lateralization towards the left side. Suppression index calculated for the right gland was <0.5, which further confirmed that the contralateral gland was suppressed. Patient was then subjected to laparoscopic left adrenalectomy. Post-surgery, his antihypertensive medication requirement decreased and potassium normalized. Histopathology report further confirmed adrenal adenoma.

3.1.3.6 Dexamethasone Androgen Suppression Test (DAST)

Aim

To differentiate the source of androgen excess (either adrenal or ovarian).

Principle

The aim of the test is to use dexamethasone, which suppresses adrenal androgen production in normal individuals but fails to do so in autonomous patients.

Indication

To identify adrenal as a source of androgen excess (virilizing adrenal tumors, CAH).

To differentiate functional ovarian vs. adrenal hyperandrogenism in PCOS.

Contraindication

There are no absolute contraindications for short or long DAST.

However, for long DAST, few relative contraindications can be seen like poorly controlled diabetes mellitus, hypokalemia, patients who are psychologically unstable, chronic kidney disease, sepsis, and life-threatening Cushing's syndrome.

Preparation

Oral formulations.

Intravenous in CKD patients.

Always ensure that appropriate medication is taken for the test performed.

Prerequisite

Patients should not be taking any enzyme-inducing (e.g., rifampicin, phenytoin) or enzyme-inhibiting drugs (e.g., fluconazole, ketoconazole).

Conditions associated with alterations in cortisol-binding globulin (pregnancy, estrogen therapy, nephrotic syndrome, hypoalbuminemia) should be ruled out prior to performing the test.

When the patient is having conditions with increased estrogen (e.g., hormone replacement therapy (HRT) or combined oral contraceptives (COCs)), then it should be stopped for at least 6 weeks prior to performing the test.

Procedure

Short DAST

It is indicated in cases with the suspected ovarian cause of hyperandrogenism.

The patient is advised to take a single tablet of 0.5 mg dexamethasone at any time of the day, and then the blood sample for testosterone and cortisol is withdrawn after 4 h.

Long DAST

It is indicated in cases with the suspected adrenal source of hyperandrogenism (virilizing tumor, CAH).

The patient is advised to 0.5 mg dexamethasone, starting at 0900 h every 6 hourly (i.e., 0900 h, 1500 h, 2100 h, and 0300 h) for 4 days (7 days if weight is over 100 kg), and then the sample is withdrawn for testosterone (or alternatively 17-hydroxyprogesterone), DHEAS, and cortisol. These samples are collected after a final dose on the following morning at 0900 h (Table 3.10).

It is important to ensure compliance to dexamethasone as per the test being done.

In cases of virilizing CAH, testosterone is usually suppressed on long DAST (<1 nmol/L), whereas testosterone from virilizing adrenal tumors is not suppressed even on long DAST.

Table 3.10 Interpretation for short and long dexamethasone androgen suppression test

Conditions		Serum testosterone	Serum cortisol
Adrenal hyperandrogenism (virilizing tumor)		>1 nmol/L or 28 ng/dL	>50 nmol/L or <50 nmol/L
PCOS	**Functional adrenal hyperandrogenism**	<1 nmol/L or 28 ng/dL	<50 nmol/L
	Functional ovarian hyperandrogenism	>1 nmol/L or 28 ng/dL	<50 nmol/L

Unit conversion

Test	Conventional unit (C)	SI unit
Serum testosterone	1 ng/dL	0.0357 nmol/L

3.1.4 Tests to Differentiate Pseudo-Cushing's from Cushing's Syndrome

3.1.4.1 Loperamide Challenge (Suppression) Test

Aim
To rule out Cushing's syndrome.

Principle
Loperamide is an opioid agonist. It has high specificity for μ receptor and decreases CRH release from the hypothalamus, which then downregulates the hypothalamic-pituitary-adrenal axis that results in suppression of cortisol in subjects with pseudo-Cushing's.

Indication: To differentiate pseudo-Cushing's versus Cushing's syndrome.

Contraindications
- Abdominal pain without diarrhea.
- Children <2 years.
- Chronic constipation.
- Bowel obstruction.
- Hypersensitivity to loperamide.

Preparation and Prerequisite
Test is performed in a fasting state.

The patient should be ready with 8 tablets of 2 mg loperamide (maximum 16 mg).

Procedure
Basal blood sample for cortisol withdrawn followed by administration of tablet loperamide of 16 mg given orally in fasting state at 8 am.

Subsequently, the blood sample is withdrawn after 210 min.

Cortisol sample is collected at baseline ($t = 0$) and after 210 min of administration of 16 mg loperamide.

Interpretation
- Serum cortisol >138 nmol/L suggestive of Cushing's syndrome.
- Serum cortisol <138 nmol/L suggestive of pseudo-Cushing's syndrome.

Complications

To watch for complications such as dizziness, abdominal cramps, constipation, and nausea.

Case 8: Loperamide Challenge Test

This is a case of 46-year-old gentleman who was a known hypertensive for 4 years with a past history of left-eye CRVO-S/P VEGF injections. He was being managed under psychiatry services for severe endogenous depression after recovering from COVID-19 illness in August 2020. He was planned for electroconvulsive therapy due to poor response to treatment. He had Cushing's stigmata in the form of round facies (Fig. 3.3a), dorsocervical fat pad (Fig. 3.3b), cuticle atrophy, and violaceous abdominal striae (Fig. 3.3c). He also had osteoporosis, dyslipidemia, and diabetes. On evaluation, he had high 11 PM cortisol (331 nmol/L) with non-suppressible ONDST (208 nmol/L) and LDDST (152 nmol/L) with a suppressible HDDST (29 nmol/L). CEMRI Sella was s/o left microadenoma (5.8 × 5.8 × 6.2 mm). In

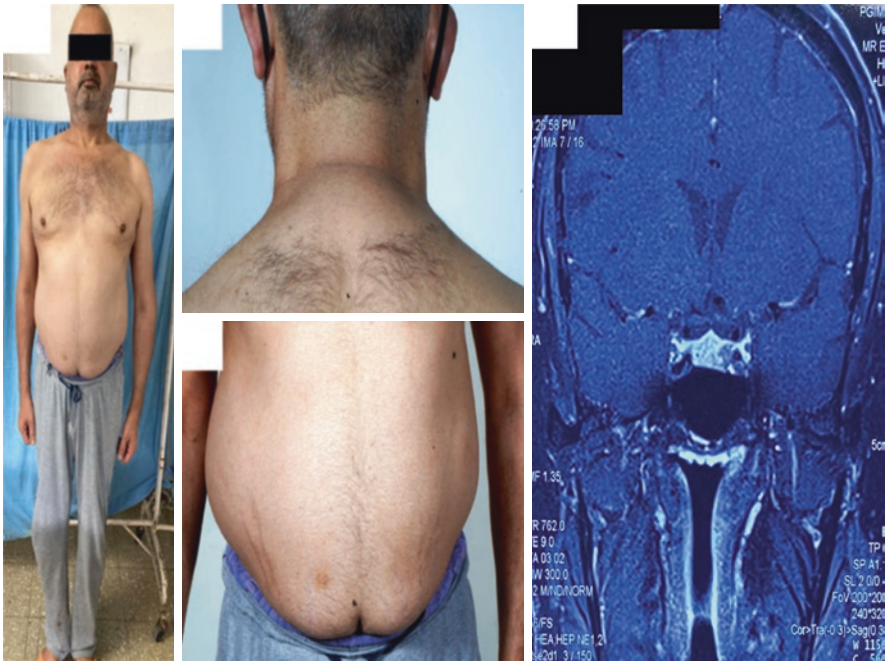

Fig. 3.3 Panel of photographs of the 46-year-old male who presented with depressive symptoms, following COVID-19 infection, requiring antidepressants. He was planned for electroconvulsive therapy. Centripetal obesity (**a**), dorsocervical fat pad (**b**), and violaceous striae (**c**) were noted. In the background of a few Cushing's stigmata and biochemical hypercortisolism with a history of antidepressant usage, loperamide test was performed to rule out pseudo-Cushing's state. A non-suppressible cortisol after loperamide (>138 nmol/L) confirmed Cushing's syndrome in the patient

order to rule out pseudo-Cushing's with an incidentaloma (Fig. 3.3d), a loperamide challenge test was done which was non-suppressible. IV ADH stimulation was done which showed 403% (28–113) rise in ACTH and 247% (370–916) rise in cortisol levels.

Clinical	Biochemical	Radiological
Striae + Facies + DC hump + Cuticular atrophy +	Cortisol 11 pm—331 ng/mL Cortisol 8 am—366 ng/mL ONDST—208 ng/mL LDDST—152 ng/mL ACTH 8 am—41.5 pg/mL	MRI s/o 5.8 × 5.9 × 6.2 mm microadenoma

3.1.4.2 16 mg Loperamide Challenge Test

	Cortisol (nmol/L)	ACTH (pg/mL)
Baseline	265	29.1
90 min	318	42.4
180 min	323	45.1
210 min	357	60.1
240 min	347	43.6

Interpretation

Inability to suppress serum cortisol to less than 138 nmol/L suggests endogenous hypercortisolemia and goes against pseudo-Cushing's state.

3.1.4.3 Low-Dose Dexamethasone Suppression Test (LDDST) Followed by CRH or Vasopressin Stimulation

Aim

To rule out Cushing's syndrome.

Principle

LDDST (oral dexamethasone 0.5 mg × 6 hourly for 48 h) suppresses the normal corticotrophs surrounding the tumor. Subsequently, intravenous administration of CRH 100 mcg or 5 IU of vasopressin stimulates the overexpressed CRH receptor on the corticotropinoma.

Indication

To differentiate pseudo-Cushing's from Cushing's syndrome.

Contraindication

There is no absolute contraindication.

However, fewer relative contraindications for LDDST like poorly controlled diabetes mellitus, hypokalemia, patients who are psychologically unstable, chronic kidney disease, sepsis, and life-threatening Cushing's syndrome need to be considered.

A L G r a w a n y

The relative contraindications for vasopressin stimulation are uncontrolled hypertension, cardiac rhythm disorders, heart failure, and suspected pheochromocytoma/paraganglioma.

Preparation

Always ensure that appropriate medication is taken for the test being performed.

Ensure that the patient is "in the cycle" (hypercortisolic) on the day of the test (in suspected cyclical Cushing's)—ideally more than 500 nmol/L.

Prerequisite

Patients should not be taking any CYP450 enzyme-inducing (e.g., rifampicin, phenytoin) or enzyme-inhibiting drugs (e.g., fluconazole, ketoconazole).

Conditions associated with alterations in cortisol-binding globulin (estrogen therapy, nephrotic syndrome, hypoalbuminemia) should be ruled out prior to performing the test.

When the patient is having conditions with increased estrogen (e.g., HRT or COC), then it should be stopped for at least 6 weeks prior to performing the test.

Procedure

LDDST is done as described below.

The cortisol is measured at 0800 h (before the first dose of dexamethasone) on the first day ($t = 0$ h) of the test and at 48 h later (6 h after the last dose) ($t = 48$ h).

Days 1 and 2: The patient takes 0.5 mg dexamethasone orally at strict 6-h intervals (i.e., 0900 h, 1500 h, 2100 h, and 0300 h) for 48 h.

The patient is advised to maintain a written record of all eight doses of dexamethasone.

Blood sample for cortisol and ACTH should be taken at 9 am on day 3, 6 h after the last dose of dexamethasone, and then vasopressin 5 IU (as available at our center) should be given intravenously slowly over 6 min (time after completion of the dose is considered as 0 in for vasopressin).

Repeat blood sample for cortisol should be taken after 15 min from time 0.

3.1.4.4 Interpretation

At 15 min:

Serum cortisol >38 nmol/L—suggestive of Cushing's syndrome.

Serum cortisol <38 nmol/L—suggestive of pseudo-Cushing's syndrome.

LDDST-CRH has PPV, NPV, sensitivity, specificity, and diagnostic accuracy of 100%.

LDDST-AVP has sensitivity of 89% and specificity of 100%.

Complications

CRH can cause flushing and hypotension, but this is rare with 100 mcg.

Vasopressin can cause abdominal pain, hypertension crisis, bradycardia, and palpitations.

3.1.4.5 Insulin-Induced Hypoglycemia (Discussed Earlier in the Chapter for GH Stimulation Test)

Aim

To rule out Cushing's syndrome.

Principle

Insulin-induced hypoglycemia activates the hypothalamo-pituitary-adrenal axis and maximally stimulates the cortisol in pseudo-Cushing's than Cushing's syndrome. Therefore, the rise in cortisol is higher in pseudo-Cushing's syndrome as compared to Cushing's syndrome.

Indication

To differentiate between Cushing's syndrome from pseudo-Cushing's syndrome.

Contraindications

Ischemic heart disease (baseline ECG in cases when clinically suspected), cardiac rhythm disorders, prior cerebrovascular accident.

Epilepsy.

Negative consent for the test.

Prerequisite/Procedure

The patient should be fasting overnight (water permitted) and remain recumbent during the test.

Regular medications can be given after completion of the test, i.e., by lunchtime.

At least two medical personnel should be present; one of them should be a physician.

Ensure availability of all materials (see consent form) required for the test and availability of emergency cart.

Two separate IV lines should be secured and patency assured by frequent heparinized saline flush [made by dissolving 25,000 U of heparin (1 vial) in 500 mL of normal saline].

Calculate regular insulin dose: 0.10 U/kg; take baseline (0 min) blood sample for cortisol, ACTH, and glucose and check blood glucose using a glucometer; administer the calculated insulin dose intravenously.

Check blood glucose with glucometer every 15 min until hypoglycemia is achieved (blood glucose <50 mg/dL). Testing for glucose can be done earlier than 15 min in case of symptoms. In case adequate hypoglycemia is not achieved even after 30 min of IV insulin administration, repeat the IV insulin dose at 0.15–0.2 IU/kg.

Adequate hypoglycemia (<50 mg/dL) should be attained, associated symptoms should be recorded, and the time of attainment of hypoglycemia needs to be considered as "30 min" from baseline. At this critical point, blood samples should be withdrawn for cortisol, ACTH (purple-top vial), and blood glucose (gray-top vial) and at 60, 90, and 120 min subsequently. During the procedure, cannula patency

should be maintained by flushing the cannula with heparinized saline between samples. Check blood glucose by glucometer every time a sample is taken.

After attainment of adequate hypoglycemia, the patient should be allowed to take orally (200–300 mL of carbonated beverage or fresh juice) and resolution of hypoglycemia should be documented after 15 min.

If symptoms of hypoglycemia are very severe or the patient is unarousable (rare) or hypoglycemia lasts for more than 30 min after an oral feed or in case of vomiting immediately after an oral feed, consider giving IV 25% dextrose (25–50 mL) and continue sampling at every 30 min. Overcorrection of hypoglycemia with intravenous dextrose needs to be avoided.

Monitor patient for 2 h (and ensure supervised meal) after completion of the test.

Interpretation

Change in serum cortisol >170 nmol/L is suggestive of pseudo-Cushing's syndrome.

Change in serum cortisol <170 nmol/L is suggestive of Cushing's syndrome.

3.1.5 Test for ACTH-Independent Macronodular Adrenal Hyperplasia (AIMAH)

AIMAH is a rare cause for Cushing's syndrome and usually diagnosed as adrenal incidentaloma or mild or subclinical Cushing's syndrome. It occurs due to the aberrant expression of one or more G-protein-coupled receptor (GPCR), which subsequently leads to proliferation and increased steroidogenesis. The aberrant receptors expressed are luteinizing hormone receptors (LH-Rs), vasoactive intestinal peptide receptors (VIPs), vasopressin receptors (i.e., V2 or V3), glucose-dependent insulinotropic peptide (GIPR), beta-adrenergic receptors, serotonin receptors, and angiotensin II receptors.

3.1.5.1 Lacroix Protocol

This protocol is developed to screen patients with adrenal Cushing's syndrome (suspected to have AIMAH), to identify the suspected regulators for steroidogenesis.

3.1.5.2 Procedure

Various tests are performed over 3–5 days in an overnight fasting state and the patient in a supine position for at least 1 h. Two IV cannulas of 18–20 G are inserted and checked for patency. Ensure patency by heparinized saline flush after every sampling [made by dissolving 25,000 U of heparin (1 vial) in 500 mL of normal saline].

3.1.5.3 On Day 1

Posture test for vasopressin, Ang-II, or catecholamine receptors.

Mixed-meal stimulation test for aberrant GIP receptor:

- *Upright posture test*: upright posture is the physiological stimuli for the vasopressin secretion and stimulates the cortisol release (Table 3.11).

3.1.6 Mixed-Meal Stimulation Test (Details Have Been Mentioned Earlier in the Chapter)

3.1.6.1 Mixed-Meal Stimulation Test

Gastric inhibitory polypeptide (GIP) is released after oral ingestion of a mixed meal that contains lipids, glucose, and proteins. These nutrients stimulate the aberrant GIP receptor expressed over the adrenal gland (hyperplastic or tumor). So, there is an increase in cAMP production and DNA synthesis in GIP-dependent cortisol-secreting tissue.

Procedure

Mixed meal (Ensure powder—non-flavored) of 2.5 g/kg dissolved in 300 mL of water to be given over 10 min. Blood sample for ACTH, and cortisol is to be taken at 0, 30, 60, 90, and 120 min (Table 3.12).

Day 2

GnRH agonist stimulation test for aberrant expression of LH/hCG receptor.

ACTH (1–24), i.e., synacthen stimulation test for aberrant ACTH receptor.

Table 3.11 Lacroix protocol (for posture testing)

Time	Test	Blood pressure	Pulse rate	ACTH	Cortisol
−60 min (0800 h)	Fasting supine (basal)			Yes	Yes
0 min (0900 h)	Upright (standing)				Yes
+30 (930 h)	Upright (standing)				Yes
+60 (1000 h)	Upright (standing)				Yes
+90 (1030 h)	Upright (standing)				Yes
+120 (1100 h)	Upright (standing)			Yes	Yes
+150 (1130 h)	Supine			Yes	Yes

Table 3.12 Lacroix protocol (for mixed-meal test)

Time	ACTH	Cortisol	Random plasma glucose (RPG)
0 min (1200 h)	Yes	Yes	Yes
+30 (1230 h)		Yes	Yes
+60 (1300 h)		Yes	Yes
+90 (1330 h)		Yes	Yes
+120 (1400 h)	Yes	Yes	Yes

3.1.6.2 GnRH Agonist Stimulation Test

Procedure

Basal sample for serum cortisol and ACTH is taken at 0 min followed by administration of GnRH agonist (injection of aqueous leuprolide or triptorelin 100 mcg) given intravenously.

Subsequently, blood samples for cortisol are withdrawn at 30, 60, 90, and 120 min (Table 3.13).

3.1.6.3 Short Synacthen Test (Details Have Been Discussed Earlier)

Procedure

Intravenously, 250 mcg of synacthen injection is given. Every 30 min, blood sampling needs to be done for cortisol and ACTH (Table 3.14).

Day 3

—

3.1.6.4 Vasopressin Stimulation Test (Discussed Earlier in Detail)

Indication: This test is performed to identify the aberrant expression of V1 or V2 receptor in response to supra-physiological stimuli of exogenous vasopressin.

Prerequisite

The test should be performed in the morning after an overnight fast.

Table 3.13 Lacroix protocol (for GnRH agonist stimulation test)

Time	Test	ACTH	Cortisol
−60 min (0800 h)	Fasting supine	Yes	Yes
0 min (0900 h)	GnRH agonist (leuprolide/triptorelin)—basal		Yes
+30 (0930 h)			Yes
+60 (1000 h)			Yes
+90 (1030 h)			Yes
+120 (1100 h)		Yes	Yes

[a] Can test testosterone and LH to know the adequacy of GnRH analog administration

Table 3.14 Lacroix protocol (for short synacthen test)

Time	ACTH	Cortisol
0 min (1100 h)	Yes	Yes
+30 (1130 h)		Yes
+60 (1200 h)		Yes
+90 (1230 h)		Yes
+120 (1300 h)		Yes

Procedure

Take blood sample for ACTH and cortisol (EDTA vial) at $T = -30$ min. Sample for ACTH to be taken separately in a pre-chilled vial and kept in a box lined with ice packs.

Patient to remain fasting and recumbent throughout the procedure.

Administer 5 IU of AVP (dilute 20 U/mL of AVP to 4 mL solution with 3 mL of normal saline and administer 1 mL of this solution) at $t = 0$, intramuscularly. Then take a blood sample for ACTH and cortisol at 0, 30, 60, and 90 min (Table 3.15).

3.1.6.5 Metoclopramide Stimulation Test

Procedure

It is used as a 5-HT4 agonist, which can be present aberrantly over adrenal tissue, stimulating the release of cortisol.

10 mg of metoclopramide is given orally.

Blood samples for ACTH and cortisol are taken at 0, 30, 60, 90, and 120 min (Table 3.16).

Interpretation

Partial Response

The rise of cortisol by 25–49% from the baseline in the absence of ACTH rise.

Positive Response

The rise of cortisol by >50% from the baseline in the absence of ACTH rise.

Table 3.15 Lacroix protocol (for vasopressin stimulation test)

Time	Test	Blood pressure	Pulse	ACTH	Cortisol
−60 min (0800 h)	Fasting supine			Yes	Yes
0 min (900 h)	Vasopressin stimulation				Yes
+30 (930 h)					Yes
+60 (1000 h)					Yes
+90 (1030 h)					Yes
+120 (1100 h)				Yes	Yes

Table 3.16 Lacroix protocol (for metoclopramide test)

Time	ACTH	Cortisol
0 min (1100 h)	Yes	Yes
+30 (1130 h)		Yes
+60 (1200 h)		Yes
+90 (1230 h)		Yes
+120 (1300 h)	Yes	Yes

Case 9: Case of AIMAH Using Lacroix Protocol

A 52-year-old female presented with worsening of glycemic and blood pressure control since 3 years, proximal muscle weakness since 1 year, and headache and blurred vision since 15 days.

Pt was diagnosed with diabetes 10 years back on routine investigations and was on oral antidiabetic agents since then. She was also on antihypertensive drugs for the past 10 years.

Since last 3 years on evaluation, it was noted that the blood glucose was uncontrolled and the patient was started on insulin two times a day along with OADs. BP was also uncontrolled, so antihypertensive medications were increased. Since 1 year, the patient started complaining of difficulty to get up from squatting position and climb stairs or comb hair, which was gradually progressive over 1 year. During this period, she also noticed a thinning of limbs and more fat accumulation in the abdomen and facial puffiness a/w with an increase in appetite. She complained of depressed mood and joint pain. On examination, she had Cushing's stigmata in the form of plethora, moon facies, centripetal obesity, dorsocervical pad of fat, supraclavicular fullness, and striae over the lower lateral abdomen. There was no hyperpigmentation. Investigations confirmed hypercortisolemia (11 pm serum cortisol of 495 nmol/L, ONDST of 477 nmol/L, LDDST of 431 nmol/L) with suppressed ACTH (1.5 pg/mL) and low DHEAS (7.8 ug/dL), confirming ACTH-independent cause of hypercortisolism. In view of multiple nodules and enlarged adrenal glands bilaterally (Fig. 3.4), a presumptive diagnosis of AIMAH was made and Lacroix protocol was performed to diagnose the specific illicit stimulator.

- Bulky right adrenal gland with macronodules.
- Bulkier left adrenal gland with multiple macronodules, largest 3.5 × 5.1 cm arising from the medial limb of the left adrenal.

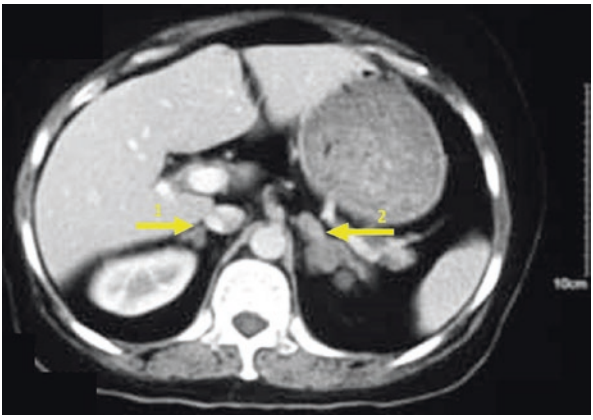

Fig. 3.4 CT adrenals of the patient showing bilateral bulky adrenal glands with multiple nodules and maintained shape

Upright posture test	ACTH	Cortisol
−60 min	1.5	555
0		510
+30		656
+60		638
+90		686
+120	1.5	717
+150	1.5	620

Mixed-meal test	ACTH	Cortisol
0 min	1.5	489
30 min		490
60		506
90		477
120	1.5	436

Triptorelin test	ACTH	Cortisol
-60 min		650
0 min		653
+30		663
+60		733
+90		749
+120	1.5	652

Metoclopramide stimulation test	21/10/21 ACTH	Cortisol
0 min	1.5	470
30 min	1.5	452
60 min	1.5	476
90 min	1.5	444
120 min	1.5	470

ADH stimulation test	ACTH (nmol/l)	Cortisol (nmol/l)
−60 min	1.5	467
0 min		534
30 min		1162
60 min		969
90 min		748
120 min	1.5	711

Acton prolongatum test	ACTH	Cortisol
0 min	14.2	697
30	730	1693
60	340	1750
90	167	1750
120	110	1750

Nasal desmopressin stimulation test	−30	−15	0	+15	+30	+60	+90	+120
ACTH	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Cortisol	550	542	570	591	575	556	558	548

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A L G r a w a n y

Endocrinology Evaluation Protocol: Gonads

4

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Contents

4.1 Gonadotropin-Releasing Hormone Agonist (GnRHa) Test 94
4.1.1 Aim 94
4.1.2 Indications 94
4.1.3 Preparation/Prerequisite 95
4.1.4 Triptorelin Stimulation Test 95
4.1.5 Leuprolide Stimulation Test 95
4.2 hCG (Human Chorionic Gonadotropin) Stimulation Test 97
4.2.1 Aim 97
4.2.2 Indications 97
4.2.3 Contraindications 98
4.2.4 Procedure 98
4.2.5 Side Effects 99
4.2.6 Conversion Factor 99
4.3 Congenital Adrenal Hyperplasia 100
4.3.1 Cosyntropin (Synacthen) Stimulation Test (ACTH [1–24]) 100
4.3.2 Aim 100
4.3.3 Indications 100
4.3.4 Prerequisite 100
4.3.5 Precautions/Contraindications 100
4.3.6 Preparation 101
4.3.7 Procedure 101
4.3.8 11β-Hydroxylase Deficiency 102
4.3.9 Interpretation 102
4.3.10 3β-HSD Deficiency 102
4.3.11 Older Criteria 102
4.3.12 Newer Criteria 102
Suggested Reading 103

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Abbreviations

21-α-OH	21-Alpha hydroxylase
3β-HSD	3-Beta hydroxysteroid dehydrogenase
ACTH	Adrenocorticotrophic hormone
CAH	Congenital adrenal hyperplasia
CDGP	Constitutional delay in growth and puberty
DHEAS	Dehydroepiandrosterone sulfate
DHT	Dihydrotestosterone
FSH	Follicle-stimulating hormone
GDPP	Gonadotropin-dependent precocious puberty
GIPP	Gonadotropin-independent precocious puberty
GnRH	Gonadotropin-releasing hormone agonist
hCG	Human chorionic gonadotropin
IGF-1	Insulin-like growth factor 1
IHH	Idiopathic hypogonadotropic hypogonadism
iPTH	Intact parathyroid hormone
LH	Luteinizing hormone
MPHD	Multiple pituitary hormone deficiency
NCCAH	Nonclassical congenital adrenal hyperplasia
OGTT	Oral glucose tolerance test
PAIS	Partial androgen insensitivity syndrome
PIH	Pregnancy-induced hypertension
PPGL	Pheochromocytoma/paraganglioma

4.1 Gonadotropin-Releasing Hormone Agonist (GnRHa) Test

4.1.1 Aim

Native gonadotropin-releasing hormone (GnRH) stimulates pituitary gonadotrophs to release gonadotropins, viz. leutinizing hormone (LH) and follicle-stimulating hormone (FSH). Gonadotropins in turn stimulate gonads to secrete gonadal steroids. This constitutes the hypothalamic-pituitary-gonadal (HPG axis). GnRHa test assesses the integrity of the HPG axis.

4.1.2 Indications

1. To differentiate between constitutional delay in growth and puberty (CDGP) vs. idiopathic hypogonadotropic hypogonadism.
2. To detect hypogonadotropic hypogonadism in patients with multiple pituitary hormone deficiency (MPHD).

3. To differentiate between gonadotropin-dependent and gonadotropin-independent precocious puberty.
4. To differentiate between gonadotropin-dependent precocious puberty and isolated premature thelarche.
5. Monitoring of children with gonadotropin-dependent precocious puberty on GnRH analogues.

4.1.3 Preparation/Prerequisite

1. Depot preparations are not used for testing.
2. Fasting is required if the test is done as a part of combined pituitary stimulation test. No fasting is required if the test is done alone.

4.1.4 Triptorelin Stimulation Test

4.1.4.1 Procedure

Collect baseline sample in EDTA vial (2 mL) between 0800 and 0900 h. Give injection of triptorelin (decapeptyl) 0.1 mg/m^2 body surface area (maximum 100 μg) subcutaneously. The sample is drawn at 3 h and 24 h after injection (Table 4.1).

Interpretation of triptorelin stimulation test for differential diagnosis of delayed puberty and precocious puberty is shown in Tables 4.2, 4.3 and 4.4 respectively.

4.1.5 Leuprolide Stimulation Test

4.1.5.1 Procedure

Collect baseline sample in EDTA vial (2 mL) between 0800 and 0900 h. Give injection of leuprolide acetate 20 μg/kg subcutaneously. Sample collection is done at baseline (0), 30, 60,120, and 180 min. 24-h sample is taken for estradiol (Table 4.5).

Table 4.1 Time schedule for triptorelin stimulation test

Baseline (0 h)	3 h	24 h
LH, FSH, testosterone/estradiol	LH, FSH	Testosterone/estradiol

Table 4.2 Triptorelin stimulation test for differential diagnosis of delayed puberty

Basal LH	Stimulated LH (3 h)	Interpretation[#]
≥0.3	≥14 mIU/L	**CDGP**
<0.3	<8 mIU/L	**Hypogonadotropic hypogonadism**
	8–14 mIU/L	**Equivocal**[a]

[a] Patient to be clinically followed up and retested after 6 months

[#] In case of discrepancy between basal and stimulated values, results are interpreted as per stimulated values

Table 4.3 Triptorelin stimulation test for differential diagnosis of precocious puberty

Basal LH	Stimulated LH (3 h)	Stimulated estradiol (24 h)	Interpretation[#]
≥0.3	≥8 mIU/L	≥80 pg/mL	**Gonadotropin-dependent precocious puberty**
<0.3	<8 mIU/L	–	**Gonadotropin-independent precocious puberty** **Premature thelarche**

[#] In case of discrepancy between basal and stimulated values, results are interpreted as per stimulated values

Table 4.4 Sensitivity and specificity of triptorelin stimulation test for gonadotropin-dependent precocious puberty

Stimulated LH (3 h)	Stimulated estradiol (24 h)	Sensitivity (%)	Specificity (%)
≥8 mIU/L	–	76	100
≥8 mIU/L	≥80 pg/mL	94	100

Table 4.5 Time schedule for leuprolide stimulation test

0 (baseline)	30 min	60 min	120 min	180 min	24 h
LH, FSH, estradiol (female)	LH, FSH	LH, FSH	LH, FSH	LH, FSH	Estradiol

Table 4.6 Leuprolide stimulation test for differential diagnosis of delayed puberty

Basal LH	Stimulated LH (3 h)	Interpretation[#]
≥0.3	≥6.1mIU/L	**CDGP**
<0.3	<2.8 mIU/L	**Hypogonadotropic hypogonadism**
	2.8–6.1 mIU/L	**Equivocal**[a]

[a]Patient to be clinically followed up and retested after 6 months
[#] In case of discrepancy between basal and stimulated values, results are interpreted as per stimulated values

Interpretation of leuprolide stimulation test for differential diagnosis of delayed puberty and precocious puberty is shown in Tables 4.6, 4.7, 4.8, and 4.9 respectively.

4.1.5.2 Side Effects

Testing with these analogs can cause nausea, headache, and, occasionally, abdominal pain.

Table 4.7 Leuprolide stimulation test for differential diagnosis of precocious puberty

Basal LH	Stimulated LH (30 min)	Stimulated LH (2 h)	Stimulated estradiol (24 h)	Interpretation[#]
≥0.3	≥9.2 mIU/L	≥5 mIU/L	≥50 pg/mL	**Gonadotropin-dependent precocious puberty**
<0.3	<4.9 mIU/L	<5 mIU/L		**Gonadotropin-independent precocious puberty Premature thelarche**
	4.9–9.2 mIU/L			**Equivocal[a]**

[a] Patient to be clinically followed up and retested after 6 months
[#] In case of discrepancy between basal and stimulated value, results are interpreted as per stimulated values

Table 4.8 Sensitivity and specificity of leuprolide stimulation test for gonadotropin-dependent precocious puberty

Stimulated LH (2 h)	Stimulated estradiol (24 h)	Sensitivity (%)	Specificity (%)
≥5 mIU/L	–	78	100
≥5 mIU/L	≥50 pg/mL	100	100

Table 4.9 Monitoring for adequacy of GnRH analog (leuprolide depot) therapy

Basal LH	Basal E2/T	Stimulated LH (3 h)
<0.6 U/L	E2 < 10 pg/mL T < 14 ng/dl	<3.3 IU/L

4.2 hCG (Human Chorionic Gonadotropin) Stimulation Test

4.2.1 Aim

LH is responsible for the production of testosterone from Leydig cells in the male. Testosterone so produced is converted to dihydrotestosterone (DHT) by enzyme 5-α reductase. Since both LH and human chorionic gonadotropin (hCG) act on the same receptor (specificity spillover), exogenous hCG is used for testing Leydig cell function.

4.2.2 Indications

1. To differentiate between partial androgen insensitivity syndrome (PAIS) and 5-α reductase deficiency.
2. To look for the presence or absence of testicular tissue (anorchia) in children with suspected bilateral cryptorchidism.

3. To differentiate between constitutional delay in growth and puberty (CDGP) vs. idiopathic hypogonadotropic hypogonadism.

4.2.3 Contraindications

No specific contraindication except hypersensitivity to hCG injection.

4.2.4 Procedure

Collect sample in EDTA vial (2 mL) between 0800 and 0900 h. Various protocols for hCG stimulation are represented in Table 4.10.

4.2.4.1 Protocol 1

A single hCG injection of 1500 IU (if age <2 years) and 5000 IU (if ≥2 years) is given intramuscularly between 0800 and 0900 h. Sample for testosterone and DHT (if done to differentiate between 5-α reductase deficiency and PAIS) is drawn at baseline and 72 h after the injection.

4.2.4.2 Protocol 2 (4-Day Test)

Injection hCG 1500 IU is given intramuscularly for 3 successive days. Sample collection for T and DHT (if done to differentiate between 5-α reductase deficiency and PAIS) is to be done at baseline and 24 h after the last injection.

4.2.4.3 Protocol 3 (7-Day Test)

Injection hCG 1500 IU is given intramuscularly on 3 alternate days. Sample collection for T and DHT (if done to differentiate between 5-α reductase deficiency and PAIS) is to be done at baseline and 48 h after the last injection.

Interpretation of hCG stimulation test in different protocols according to clinical condition for which test was performed, which is represented in Tables 4.11, 4.12, 4.13, and 4.14.

Table 4.10 Time schedule for different protocols of hCG stimulation test

	Protocol 1	Protocol 2 (4-day test)	Protocol 3 (7-day test)
Regimen	Single hCG injection 1500 IU (age <2 years) 5000 IU (age ≥2 years)	1500 IU of hCG injection im x 3 days (D1, D2, D3)	1500 IU of hCG injection im on 3 alternate days (D1, D3, D5)
Sampling	D1 (basal) D4	D1 (basal) D4	D1 (basal) D7

Table 4.11 hCG stimulation test (protocol 1 and protocol 2) for the differential diagnosis of delayed puberty

Basal testosterone	Delta T	Interpretation[#]
≥0.7	≥9 nmol/L	**CDGP**
<0.7	<3 nmol/L	**HH**
	3–9 nmol/L	**Equivocal**[a]

[a] Patient to be clinically followed up and retested after 6 months

In case of discrepancy between basal and stimulated value, results are interpreted as per stimulated values

Table 4.12 hCG stimulation test (protocol 3) for the differential diagnosis of delayed puberty

Stimulated T	Interpretation
≥8 nmol/L	**CDGP**
<8 nmol/L	**HH**

Table 4.13 hCG stimulation test for differential diagnosis of bilateral cryptorchidism (only for protocol 2)

Stimulated T	Interpretation
≥150 ng/dl (5.21 nmol/L)	Presence of testicular tissue
<150 ng/dl (5.21 nmol/L)	Absence of testicular tissue/ Leydig cell dysfunction

Table 4.14 hCG stimulation test for differentiation of 5-α reductase deficiency and partial androgen insensitivity syndrome (Protocol 1/ Protocol 2/Protocol 3)

Stimulated T/DHT ratio (nmol/L)	Interpretation
≥10	**5-α reductase deficiency**
<10	**Partial androgen insensitivity syndrome (PAIS)**

4.2.5 Side Effects

Testing with these agents can result in pain abdomen, nausea, vomiting, and pain/redness at the injection site.

4.2.6 Conversion Factor

Testosterone: (ng/mL) × 3.467 = nmol/L.

Testosterone: (ng/dl) × 0.0347 = nmol/L.

DHT: (ng/dl) × 0.0344 = nmol/L.

4.3 Congenital Adrenal Hyperplasia

4.3.1 Cosyntropin (Synacthen) Stimulation Test (ACTH [1–24])

4.3.2 Aim

Synacthen has the same physiological action as endogenous adrenocorticotropic hormone (ACTH). In the normally functioning adrenal cortex, it stimulates the biosynthesis of glucocorticoids and androgens.

4.3.3 Indications

1. For diagnosis of classical congenital adrenal hyperplasia (CAH) (21-α-OH deficiency).
2. To differentiate classical CAH and nonclassical congenital adrenal hyperplasia (NCCAH).
3. To differentiate NCCAH and polycystic ovarian disease (PCOS).
4. For diagnosis of CAH other than 21-α-OH deficiency.
5. For diagnosis of adrenal insufficiency (covered elsewhere).

4.3.4 Prerequisite

- Deferred until after the first 72 h of life.
- Done in the follicular phase (female).

4.3.5 Precautions/Contraindications

- Hypersensitivity to tetracosactide and/or ACTH of animal origin or to any component of the formulation.
- Heart failure (refractory).
- Pregnancy (category D).

4.3.5.1 Adverse Reactions

1. *Hypersensitivity reactions:* Hypersensitivity reactions may include skin reactions at the injection site, dizziness, nausea, vomiting, urticaria, flushing, malaise, dyspnea, and angioneurotic edema.
2. *Adrenal hemorrhage:* Rare.

4.3.6 Preparation

- Synacthen is a long-chain polypeptide composed of the first 24 of the 39 amino acids contained in the naturally occurring ACTH (corticotrophin) molecule. Synacthen 250 micrograms correspond to 25 IU of ACTH.
- Active ingredient: Tetracosactide.
- Injection: 250 micrograms/mL, 1 mL ampoule.
- Storage: protect from light and store in a refrigerator (2-8 °C).

4.3.7 Procedure

- Injection of synacthen 250 μg (ACTH [1–24]) is given intravenously. In children <1 year with low birth weight, 125 μg can be given (intramuscularly if no IV access).
- Samples are collected for cortisol and 17-OHP (17-hydroxy progesterone) at baseline; sample to be collected between 0800 and 0900 h (if not previously done) and at 60 min.
- Ideally, samples are collected for 17-OHP, cortisol, 11-deoxycorticosterone, 11-deoxycortisol, 17-OH pregnenolone, dehydroepiandrosterone, and androstenedione by LC-MS.
- Interpretation of cosyntropin test for differential diagnosis of congenital adrenal hyperplasia is shown in Tables 4.15 and 4.16.
- However, salt wasting is now based on clinical and biochemical parameters.

Table 4.15 Cosyntropin test for differentiation of classical congenital adrenal hyperplasia, nonclassical CAH, and polycystic ovarian syndrome

Stimulated 17-OHP	Interpretation
<2 ng/mL	Congenital adrenal hyperplasia (CAH) excluded
2–10 ng/mL	CAH other than 21-α-OH deficiency Congenital adrenal hyperplasia (heterozygous) Polycystic ovarian syndrome
10–100 ng/mL	Nonclassical CAH (21-α-OH deficiency)
>100 ng/mL	Classical CAH (21-α-OH deficiency)

Table 4.16 Cosyntropin test for differential diagnosis of classical congenital adrenal hyperplasia

Classical CAH	Interpretation
100–500 ng/mL	Simple virilizing (21-α-OH deficiency)
>500 ng/mL	Salt wasting CAH (21-α-OH deficiency)

Table 4.17 Cosyntropin test for diagnosis of congenital adrenal hyperplasia (3β-HSD deficiency)

Population being tested	Stimulated 17-OH-pregnenolone level
Neonates with ambiguous genitalia	≥378 nmol/L
Tanner stage I children with ambiguous genitalia	≥165 nmol/L
Children with premature pubarche	≥294 nmol/L
Adults	≥289 nmol/L

4.3.8 11β-Hydroxylase Deficiency

Basal (if not previously done) and ACTH stimulated 11-deoxycortisol and DOC (11-deoxy corticosterone) levels 1 h after synacthen injection.

4.3.9 Interpretation

Levels three times above the 95th percentile for the normal population are considered diagnostic for 11β-hydroxylase deficiency.

4.3.10 3β-HSD Deficiency

Basal (if not previously done) and ACTH stimulated 17-OH-pregnenolone and DHEAS levels 1 h after synacthen injection.

4.3.11 Older Criteria

More than 2 standard deviation (SD) values above the normal mean value for 17-OH-pregnenolone and DHEAS levels were considered diagnostic for 3β-HSD deficiency.

4.3.12 Newer Criteria

Diagnostic cutoffs of 17-OH-pregnenolone for the diagnosis of 3β-HSD deficiency after cosyntropin stimulation test in different populations are represented in Table 4.17.

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Endocrinology Evaluation Protocol: Bone and Mineral Disorders

5

Rimesh Pal and Sanjay Kumar Bhadada

Contents

5.1 Renal Tubular Acidosis..... 106
5.1.1 Ammonium Chloride Loading Test..... 106
5.2 Bicarbonate Loading Test..... 108
5.2.1 Principle..... 108
5.2.2 Indication..... 109
5.2.3 Patient Preparation..... 109
5.2.4 Protocol..... 109
5.2.5 Interpretation..... 109
5.3 Measurement of TmP/GFR..... 111
5.3.1 Principle..... 111
5.3.2 Indications..... 111
5.3.3 Protocol..... 111
5.3.4 Calculation..... 111
5.3.5 Interpretation..... 112
Suggested Reading..... 114

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5.1 Renal Tubular Acidosis

5.1.1 Ammonium Chloride Loading Test

5.1.1.1 Principle

Ammonium chloride is converted in the liver to urea with the utilization of bicarbonate. This leads to a reduction in plasma bicarbonate and induction of metabolic acidosis. In a healthy individual, this leads to renal synthesis of bicarbonate and excretion of hydrogen ions (H^+) in urine to maintain normal blood pH. However, in patients with distal (type 1) renal tubular acidosis (RTA), excretion of hydrogen ions fails to occur, thereby leading to unmasking of metabolic acidosis and alkaline urine (urine pH > 5.5).

5.1.1.2 Indication

The diagnosis of distal RTA is confirmed in a patient with normal anion gap metabolic acidosis with normal renal function and urine pH > 5.5. However, a patient clinically suspected of having distal RTA with no evidence of metabolic acidosis on arterial blood gas analysis will require an ammonium chloride loading test for the confirmation of diagnosis of distal RTA. This is usually seen in patients who have incomplete or mild forms of distal RTA.

5.1.1.3 Contraindications

- It should NOT be performed if the urine is alkaline in the presence of metabolic acidosis (as in an obvious case of distal RTA) or in metabolic acidosis due to any obvious cause (like renal failure).
- It is contraindicated in patients with liver disease.
- This test should not be performed in hypokalemia or hypercalcemia as these conditions interfere with tubular function and may mimic RTA.

5.1.1.4 Patient Preparation and Prerequisite

- Bacterial urinary tract infection must be ruled out as it may give a falsely high urine pH due to urea hydrolysis.
- The patient should be nil by mouth for 8 h preceding the test.

5.1.1.5 Protocol

- The test should be performed under close supervision.
- 5 mL of venous blood is collected in a heparinized vial prior to administration of ammonium chloride for estimation of plasma bicarbonate at baseline.
- Ammonium chloride (100 mg/kg body weight) is given orally with breakfast mixed with syrup or jam. The total dose should be taken over a period of 2 h to reduce the degree of gastric irritation. A single dose of 75 mEq/m^2 of ammonium chloride in infants administered via nasogastric tube or 150 mEq/m^2 in children over 1 h in gelatin-coated capsules is usually given.

- If the total dose cannot be consumed, the exact quantity ingested should be noted down. It will be necessary to repeat test if the patient vomits all/majority of the ammonium chloride.
- Urine samples are collected every hour for 6 h. Each urine sample should be sent immediately to the concerned laboratory for pH measurement.
- After 6 h of completion of ingestion of ammonium chloride, another sample of venous blood is collected for re-estimation of plasma bicarbonate.

5.1.1.6 Interpretation

Normal response: The urine pH should fall to <5.2 in at least one specimen, associated with a decline in plasma total bicarbonate by 3–5 mEq/L.

In patients with distal RTA, the urine pH remains above 6.0 indicating an inability to acidify the urine in spite of a low plasma bicarbonate concentration.

In patients with proximal RTA (type 2) and those with generalized renal tubular damage, the urine pH falls to <5.2 at some point during the test, i.e., a normal response.

This test does not, however, differentiate satisfactorily between these two forms of renal tubular acidosis.

5.1.1.7 Comments

- In patients with liver disease, calcium chloride may be used as an acidifying agent at a dose of 2 mEq/kg body weight.

Case 11: Ammonium Chloride Test

A 14-year-old male with a history of poor height and weight gain since childhood noticed around 7 years of age, now presented with a history of polyuria to the endocrinology outpatient clinic. There was no history of polydipsia or salt craving. There was no history of chronic headache or visual deficits. There were no skeletal deformities or dysmorphic facies on examination.

On evaluation, biochemistry revealed normal glycemic profile, normal serum sodium and calcium levels, but low potassium levels. Hormonal profile and urine/serum osmolality were normal. Ultrasound of the kidneys was normal. Radiograph of the wrist was suggestive of rickets (Fig. 5.1).

In view of short stature, polyuria, and hypokalemia, possibility of renal tubular acidosis was kept. Arterial blood gas analysis done multiple times was suggestive of variable HCO_3 levels varying from 18 to 22 meq/L with urine pH above 5.5, raising the possibility of renal tubular acidosis. There was no evidence of phosphaturia, proteinuria, or glucosuria. Ammonium chloride testing was done to confirm the possibility of RTA. The values are represented below:

Body weight of the child: 26 kg.

Dose of ammonium chloride administered (100 mg/kg): 2.6 g.

Fig. 5.1 X-ray of wrists of the index patient showing classical radiological features of rickets, including metaphyseal widening (splaying) and fraying at bilateral wrist joints

	9 am	11:30 am	12:30 pm	1:30 pm	2:30 pm	3:30 pm	4:30 pm
Urine pH	6.5	6.5	6.5	6.5	6.5	6.5	6.2
VBG parameters							
pH	7.40						7.296
PCO_2	36.6						45.3
PO_2	77.2						31.6
HCO_3	22						16.6
Lactate	1.50						1.42

Following ammonium chloride loading, the plasma bicarbonate came down to 16.6 mEq/L; however, the urine pH remained above 6, confirming the diagnosis of RTA, likely distal RTA.

5.2 Bicarbonate Loading Test

5.2.1 Principle

The principle of the bicarbonate loading test is that in subjects with acidemia, low plasma bicarbonate concentration, and intact proximal tubule function, administering bicarbonate should not alter urine bicarbonate levels because the bicarbonate is avidly reabsorbed from the urine. However, in patients with proximal RTA, urinary

bicarbonate concentration will rise following bicarbonate infusion because of impaired reabsorption from the proximal tubule.

5.2.2 Indication

- Characterization of the type of RTA (distal vs. proximal).

5.2.3 Patient Preparation

The patient should be nil by mouth for at least 8 h preceding the test.

5.2.4 Protocol

- This test is usually performed by administering 4 mEq/kg of oral sodium bicarbonate (1 g = 12 mEq).
- This dose, however, does not normalize plasma HCO_3^- in a large proportion of patients with marked HCO_3^- wasting, such as those with proximal RTA. These patients require larger doses of oral bicarbonate or intravenous infusion of a 3.75% solution of sodium HCO_3^- at rates varying from 0.3 to 0.8 mL/min to cause an increment of 2–3 mEq/L/h of plasma HCO_3^- and to minimize extracellular volume expansion.
- Simultaneously, urine pH is measured in timed samples taken 30–60 min apart. The urine should ideally be collected under mineral oil, with the child voiding in the upright position.
- A steady state is usually achieved after 3 to 4 h of the start of infusion, and the test is terminated when three samples of timed urine collections show urine pH >7.5.
- Thereafter, a venous blood sample is collected for the estimation of plasma bicarbonate and plasma creatinine.

5.2.5 Interpretation

5.2.5.1 Fractional Excretion of Bicarbonate ($FEHCO_3^-$)

This test allows the calculation of the fractional excretion (FE) of HCO_3^- ($FEHCO_3^-$). FE of HCO_3^- (expressed as %) is determined by using the formula (urine HCO_3^- × plasma creatinine × 100) / (plasma HCO_3^- × urine creatinine). This is an important index of proximal tubular handling of HCO_3^-. The $FEHCO_3^-$ is calculated after adequate alkalization. The proximal tubule normally reabsorbs almost all filtered bicarbonate (fractional excretion <5%).

When the serum bicarbonate is normal (>22 mEq/L), a value >15% indicates proximal RTA. Levels are usually in the normal range (<5%) in classic distal RTA.

5.2.5.2 Urine-to-blood (U-B) pCO_2 gradient

In alkaline urine, such as after a load of sodium bicarbonate, urine pCO_2 increases because of distal H^+ secretion and is considered a sensitive indicator of distal acidification. The secreted H^+ in the distal tubule reacts with luminal HCO_3^- to form carbonic acid, which dehydrates slowly in the medullary collecting duct to form CO_2 that is trapped in the renal tubule. If urine pH is >7.5 and plasma HCO_3^- concentration >23–25 mEq/L, the urine pCO_2 should exceed 70 mmHg and the U-B pCO_2 gradient should be greater than 20 mmHg in normal individuals. Patients with decreased rates of tubular H^+ secretion (classical type 1 RTA) show subnormal values, with urine pCO_2 less than 50 mmHg and U-B pCO_2 <10 mmHg.

Case 12: Fractional Excretion of Bicarbonate

A 13-year-old girl presented with acute flaccid paralysis to emergency; when on evaluation, she was found to have hypokalemia (serum potassium = 1.3 mEq/L), which improved on parenteral potassium supplementation. She had a similar episode 2 years back. There was no history of preceding infection, strenuous exercise, or intake of carbohydrate-rich meal. There was no similar family history.

Besides hypokalemia, arterial blood gas analysis was suggestive of normal anion gap metabolic acidosis.

pH	HCO_3^-	K^+	Na^+	Cl^-
7.123	16.8	1.23	136	112

Hypokalemia and normal anion gap metabolic acidosis were suggestive of RTA. Her urine pH was 5. Points against distal RTA were absence of nephrolithiasis/nephrocalcinosis and mild proteinuria on 24-h urinary collection (167 mg/total volume). Nevertheless, there were no other manifestations of proximal RTA like hypophosphatemia, phosphaturia, or glycosuria.

To confirm the diagnosis, oral bicarbonate loading test was done (4 mEq/kg):

$$\begin{aligned}\text{FEHCO}_3^- &= (\text{Urine HCO}_3^- \times \text{Plasma creatinine} \times 100) \\ &\quad / (\text{Plasma HCO}_3^- \times \text{Urine creatinine} \times 100) = 0.6\%\end{aligned}$$

- As $FEHCO_3^-$ was less than 5%, it confirmed the diagnosis of distal RTA.

5.3 Measurement of TmP/GFR

5.3.1 Principle

The ratio of tubular maximum reabsorption of phosphate (TmP) per unit volume of glomerular filtration rate (GFR) is used to evaluate renal phosphate transport. This essentially corresponds to the theoretical lower limit of serum phosphate below which all filtered phosphate would be reabsorbed.

Calculation of TmP/GFR assumes that serum phosphate concentration is equal to its concentration in the glomerular filtrate and that creatinine clearance is a close approximation to GFR.

5.3.2 Indications

It is used to establish renal phosphaturia in a variety of pathological conditions associated with hypophosphatemia like

- X-linked hypophosphatemic rickets
- Tumor-induced osteomalacia
- Autosomal dominant hypophosphatemic rickets
- Autosomal recessive hypophosphatemic rickets
- Hereditary hypophosphatemic rickets with hypercalciuria

5.3.3 Protocol

- Overnight fast.
- The first voided urine should be discarded.
- Collect a 25 mL sample of the second void urine into a universal container for urine creatinine and phosphate.
- A serum sample should be collected at the same time for serum creatinine and phosphate.
- Both the urine and serum samples should be sent to the laboratory for analysis.

5.3.4 Calculation

1. Calculate the ratio of phosphate clearance to creatinine clearance (CP/CCr)

$$\text{CP/CCr} = (\text{Serum creatinine} \times \text{Urine phosphate}) / (\text{Urine creatinine} \times \text{Serum phosphate})$$

(This ratio is normally less than 0.15).

2. Subtract this fraction from 1.0 to give the fractional tubular reabsorption of phosphate (TRP).

$$\text{TRP} = 1 - (\text{Serum creatinine} \times \text{Urine phosphate})\ (\text{Urine creatinine} \times \text{Serum phosphate})$$

3. If TRP is < 0.86, then phosphate reabsorption is maximal and there is a linear relationship between plasma phosphate concentration and excretion and TmP/GFR which is calculated by:

$$\text{TmP / GFR} = \text{TRP} \times \text{serum phosphate}$$

4. If TRP is > 0.86 relationship between plasma phosphate concentration and excretion is curvilinear and TmP/GFR is defined as follows:

$$\text{TmP / GFR} = \alpha \times \text{serum phosphate, where } \alpha = \left(0.3 \times \text{TRP}\right) / 1 - \left(0.8 \times \text{TRP}\right)$$

Alternatively, TmP/GFR can also be determined using a nomogram after measuring fasting plasma and urine concentrations of phosphate and creatinine.

Walton-Bijvoet nomogram is used to determine tubular maximum reabsorption rate of phosphate to glomerular filtration rate (TmP/GFR) given plasma phosphate (PO_4) concentration and tubular reabsorption of phosphate (TRP).

On the vertical axes, the inner scale is in mmol/L, whereas the outer scale is in mg/dL. TRP is calculated as detailed in text. A straight line joining plasma phosphate concentration, TRP, and right vertical axis gives the TmP/GFR.

5.3.5 Interpretation

Low levels suggest renal phosphate wasting.

Age-related reference ranges for TmP/GFR should be used for comparison.

Case 13: TmP/GFR Estimation

A 33-year-old gentleman presented with complaints of low backache for 4 years, along with proximal muscle weakness of both lower limbs, which was progressive, such that he needed assistance for walking after 2 years. In addition, he had sustained right forearm and bone fragility fractures 1 year back.

On evaluation for the abovementioned complaints, his biochemistry was significant for the following findings:

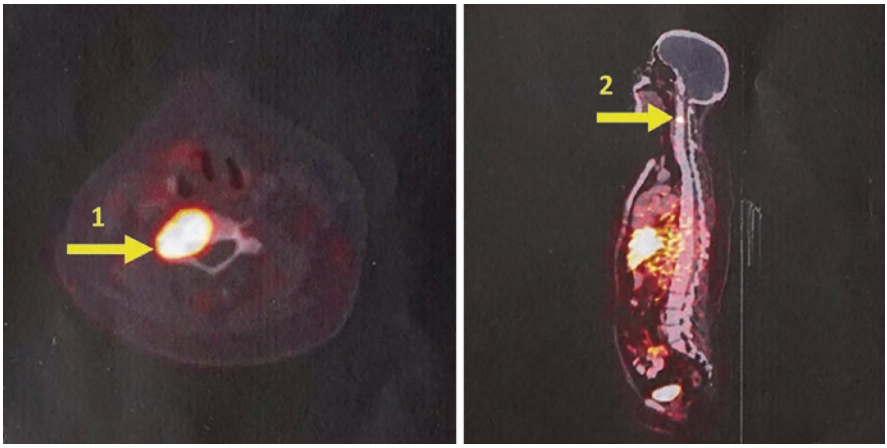

Fig. 5.2 Panel of images depicting the somatostatin receptor-based scintigraphy images of a patient with biochemical profile suggestive of hypophosphatemia and phosphaturia with elevated FGF23, consistent with tumor-induced osteomalacia. The images depict an SSTR-expressing lesion in C4 vertebra

Calcium (mg/dL)	Phosphorus (mg/dL)	Albumin (gm/dL)	ALP (IU/L)	25(OH)D (ng/mL)	PTH (pg/mL)
9.0	1.3	4	306	70	70.49

In view of hypophosphatemia, TRP and TmP/GFR were calculated as follows:
Plasma phosphate (Pp) 1.3 mg/dL.
Urine phosphate (Up) 1100 mg.
Plasma creatinine (Pcr) 0.6 mg/dL.
Urine creatinine (Ucr) 1538 mg.
TRP = 1 − {(Up/Pp) × (Pcr/Ucr)}.
= 0.67.
As TRP was less than 0.86,
TmP/GFR = TRP × Pp.
= 0.67 × 1.3.
= 0.87 mg/dL
Reference Range for TmP/GFR in Adults 2.7–4.5 mg/dL

Both serum phosphate and TmP/GFR were low, suggestive of renal phosphate wasting. Arterial blood gas analysis was normal.

Further, FGF23 was measured using C-terminal ELISA assay which was elevated at 356 RU/mL (reference range 0–150 RU/mL). Hence, a possibility of tumor-induced osteomalacia was considered. Functional imaging using somatostatin receptor (SSTR)-based scintigraphy was performed that was suggestive of an SSTR-expressing lesion in C4 vertebra (SUVmax 17.2) (Fig. 5.2).

A L G r a w a n y

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Endocrinology Evaluation Protocol: Special Situations

6

Shakun Chaudhary, Liza Das, and Soham Mukherjee

Contents

6.1 Diagnosis of Endocrine Disorders in Special Circumstances..... 115
6.1.1 Cushing's Syndrome..... 115
6.1.2 Pheochromocytoma..... 117
6.1.3 Primary Hyperparathyroidism..... 119
6.1.4 Acromegaly..... 121

6.1 Diagnosis of Endocrine Disorders in Special Circumstances

6.1.1 Cushing's Syndrome

6.1.1.1 Pregnancy

Diagnostic Challenges

- Urinary free cortisol (UFC) excretion is normal in the first trimester; however, it increases up to threefold by term to overlap with values seen in women with Cushing's syndrome. So, screening for hypercortisolism is difficult in the second and third trimesters.
- Serum cortisol circadian variation is preserved in normal pregnancy, albeit with a higher midnight nadir. Hence, cutoffs used in non-pregnant female for loss of circadian rhythm cannot be used in pregnancy.

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- Dexamethasone suppression test is not recommended as it can yield false-positive result due to estrogen-mediated elevation of cortisol-binding globulin.

Diagnosis

Diagnostic criteria for diagnosis of Cushing's syndrome in pregnancy are represented in Table 6.1.

Instructions for Urinary Free Cortisol Sample Collection

- The first morning void is discarded so that the collection begins with an empty bladder. All subsequent voids throughout the day and night should be included in the collection, which is kept refrigerated (but not frozen).
- Patients should be instructed not to drink excessive amounts of fluid and to avoid the use of any glucocorticoid preparations, including steroid-containing skin or haemorrhoid creams.

Tumour Localisation

MRI without contrast is the preferred modality of imaging.

6.1.1.2 Chronic Kidney Disease

Diagnostic Challenges

- Excreted urine cortisol values decrease below creatinine clearance of 60 mL/min/1.73 m^2 and are quite low, below 20 mL/min/1.73 m^2.
- Serum cortisol values measured over a 24-h period are reported to be elevated.
- Diagnostic thresholds for either serum or salivary cortisol are not known.
- The absorption and metabolism of 1 mg dexamethasone, as well as the cortisol response, have been reported to be both normal and abnormal.
- Plasma adrenocorticotrophic hormone (ACTH) is increased in chronic kidney disease (CKD), possibly due to increased stimulation of the hypothalamic-pituitary-adrenal axis by the hyperinflammatory state. Hence, differentiation between ACTH-dependent and ACTH-independent Cushing's syndrome becomes difficult.
- Various parameters used for the diagnosis of Cushing's syndrome can be deranged in CKD patient even without Cushing's syndrome as shown in Table 6.2.

Table 6.1 Diagnostic criteria for diagnosis of Cushing's syndrome in pregnancy

Test	Diagnostic criteria
Urinary free cortisol	>Three times upper limit of normal (ULN) reference range for non-pregnant in second/third trimester[a]
Urinary free cortisol	>ULN (non-pregnant) in first trimester[a]

[a] At least two collections of urinary free cortisol should be performed

Table 6.2 Alteration in cortisol dynamics in CKD patient without Cushing's syndrome

Parameter	CKD patient without Cushing's syndrome
Morning serum cortisol	Increased
24-h UFC	Decreased
Circadian rhythm Cortisol ACTH	Preserved[a]
Oral dexamethasone suppression test ONDST (1 mg)/LDDST (4 mg) HDDST (8 mg)	Non-suppressed (≥50 nmol/L) Suppressed (<50 nmol/L)

[a]Loss of circadian rhythm can sometimes occur in CKD, particularly in patients receiving dialysis

Diagnosis

- There are no validated diagnostic criteria.
- Failure to suppress cortisol to <50 nmol/L in response to a high-dose dexamethasone (8 mg) suppression test suggests diagnosis of Cushing's syndrome.
- Loss of circadian rhythm of both cortisol and ACTH suggests diagnosis of Cushing's syndrome.
- Normal response to 1 mg dexamethasone excludes Cushing's syndrome, but an abnormal response is not diagnostic. Because of altered absorption and metabolism of dexamethasone, measurement of serum dexamethasone concentration should be done to ensure adequate bioavailability.
- Intravenous dexamethasone suppression test can also be used, but has not been validated in CKD patients so far.

Tumour Localisation

MRI sella and CT abdomen without contrast depending upon clinical and biochemical suspicion of ACTH-dependent and ACTH-independent Cushing's syndrome.

6.1.2 Pheochromocytoma

6.1.2.1 Pregnancy

Diagnostic Challenges

- Presentation of pheochromocytoma paraganglioma (PPGL) in pregnancy is similar to the non-pregnant state. However, the disease remains underdiagnosed because of the higher prevalence of pregnancy-induced hypertension and lesser suspicion of PPGL.
- Catecholamine levels do not rise in the normal healthy pregnancy. However, pregnancy-induced hypertension can result in mild increase in catecholamine. Important clues in the favour of PPGL and pregnancy-related hypertension are shown in Table 6.3.

Table 6.3 Characteristics of PPGL and pregnancy-related hypertension

PPGL more likely than pregnancy-related hypertension	Presentation of hypertension <20 weeks of gestation
	Paroxysmal hypertension
	Paroxysmal headache
	Orthostatic hypotension
	Syndromic features
Pregnancy-related hypertension more likely than PPGL	Proteinuria
	Oedema
	HELLP (haemolysis, elevated liver enzymes and low platelet count)

Diagnosis

- Biochemical testing for a PPGL in pregnant patients is not different from non-pregnant patients, although there are no clinical studies that have assessed the accuracy of biochemical tests or established specific reference values in a pregnant population.
- Measurement of plasma and/or 24-h urine metanephrine and normetanephrine concentrations is used for diagnosis similar to non-pregnant individuals.

False-Positive Result

- Labetalol or methyldopa in some LC-electrochemical detection assays may lead to false-positive yields but not in LC-MS/MS assays.
- Inappropriate sampling in the sitting (30% higher plasma normetanephrine values) instead of recommended supine position for at least 20 min.

False-Negative Result

- Improper/inadequate collection.
- Non-functional tumour.

Tumour Localisation

- After the biochemical diagnosis is made, tumour localisation should be done by either ultrasonography or MRI.
- MRI without gadolinium has high accuracy in detecting pheochromocytoma in pregnancy.
- CT scan and MIBG scan are avoided during pregnancy.

6.1.2.2 Chronic Kidney Disease

Diagnostic Challenges

- Many patients with ESRD are hypertensive due to their renal disease leading to decrease in clinical suspicion.
- The usual screening test involving a 24-h urinary collection for metanephrines and catecholamines is not recommended in anuric/oliguric patients.

- Serum norepinephrine concentrations are elevated two to three times in CKD with or without haemodialysis, while epinephrine concentrations remain unchanged.
- Major mode of excretion for vanillylmandelic acid (VMA) is renal. So, measurement of VMA is not useful in CKD. Kidneys make only minor contribution to the clearance of free metanephrines (14–16%) and circulating catecholamines (15–24%). Hence, measurement of free metanephrines and catecholamines is the method of choice.

Diagnosis
- Normal results for plasma-free metanephrines in the setting of CKD rule out pheochromocytoma.
- Increase in plasma norepinephrine higher than threefold above normal and/or epinephrine higher than the upper limit of normal can be considered suggestive of pheochromocytoma.
- Role of plasma-free metanephrines higher than threefold above normal for the diagnosis of pheochromocytoma is controversial.
- False-positive results can occur due to influences of sympathetic activation and unrecognised analytical interferences. Combination of positive tests or repeat testing of plasma-free metanephrines using a different laboratory and method of analysis should be done for the confirmation of diagnosis of pheochromocytoma.

Tumour Localisation
CT abdomen without contrast is the preferred imaging modality.

6.1.3 Primary Hyperparathyroidism

6.1.3.1 Pregnancy

Diagnostic Challenges
- Most of the pregnant patients of primary hyperparathyroidism (PHPT) are either asymptomatic or may have mild non-specific symptoms, which are similar to symptoms associated with normal pregnancy.
- Changes during normal pregnancy in calcium homeostasis result in relatively lower level of serum total calcium.
- Factors responsible for relatively lower total calcium level in pregnancy include haemodilution, hypoalbuminaemia, trans-placental calcium transport to the foetus and hypercalciuria resulting from an increased glomerular filtration rate. Moreover, oestrogen may also inhibit PTH-induced bone resorption.

Diagnosis
- Serum calcium value higher than the normal reference range with elevated PTH with normal 25(OH)D levels should be investigated for PHPT.

- A high index of suspicion for PHPT should be kept for women who have excessive pregnancy-associated symptoms.
- In the setting of clinical suspicion, even serum total calcium at the upper normal level should be evaluated further.

Tumour Localisation

- Neck ultrasonography is the safest modality in pregnancy and has a sensitivity of approximately 70%.
- The ^{99m}Technetium sestamibi scintigraphy is contraindicated during pregnancy.

6.1.3.2 Chronic Kidney Disease

Diagnostic Challenges

Chronic renal failure is associated with parathyroid hormone (PTH) resistance resulting in secondary hyperparathyroidism. So, moderate rise in PTH level in a CKD patient is not suggestive of autonomous secretion. PTH levels according to the stage of CKD are shown in Table 6.4.

Diagnosis

Presence of hypercalcaemia/high normal calcium along with high PTH level in the setting of CKD is suggestive of autonomous secretion of PTH, i.e. primary or tertiary hyperparathyroidism and warrants further evaluation.

Clinical and biochemical parameters indicating primary hyperparathyroidism or tertiary hyperparathyroidism are shown in Table 6.5.

Tumour Localisation

- Neck ultrasonography and ^{99m}Technetium sestamibi scintigraphy are used for tumour localisation.

Table 6.4 PTH levels in various stages of CKD

Stage of CKD	eGFR range (mL/min/1.73 m^2)	PTH (pg/mL)
1	>90	Normal range
2	60–89	Normal range
3	30–59	35–70
4	15–29	70–110
5	<15 or on dialysis	150–300

Table 6.5 Clinical and biochemical parameters in primary hyperparathyroidism vs. tertiary hyperparathyroidism

Primary hyperparathyroidism	Tertiary hyperparathyroidism
Short duration of CKD	Long duration of CKD
Presence of renal stone disease	Patient on haemodialysis
Syndromic features	
Hypercalcaemia with hypophosphataemia	Hypercalcaemia/high normal calcium with normophosphataemia/hyperphosphataemia

6.1.4 Acromegaly

6.1.4.1 Pregnancy

Diagnostic Challenges

- During early pregnancy, pituitary growth hormone (GH) increases because of the stimulatory effect of oestrogen. However, after the 25th week of pregnancy, pituitary GH decreases. Placental GH starts appearing in maternal circulation at 10 weeks and peaks at 25–27 weeks. Therefore, the measurement of GH and GH-GTT (GH-glucose tolerance test) is not helpful in pregnancy.
- In a normal pregnancy, the physiological increase in oestrogen and progesterone decreases the sensitivity of the liver. This can result in modest reduction in circulating insulin-like growth factor (IGF-1) in early pregnancy. So, IGF-1 can be normal even in the setting of acromegaly in early pregnancy.
- IGF-1 increases two- to threefold in the second half under the influence of placental GH and human placental lactogen (HPL) with a peak at around 37 weeks. So, IGF-1 is high even in the second half of normal pregnancy making diagnosis of acromegaly difficult.

Diagnosis

Definite diagnosis should be postponed (if possible) until the post-partum period due to lack of normative data during pregnancy. However, probable diagnostic criteria are the following:

- A high serum IGF-1 level before mid-gestation with features of acromegaly.
- Post-glucose GH levels ≥1 μg/L by highly specific assays for pituitary GH during third trimester.

Tumour Localisation

- MRI without contrast is the preferred modality of imaging.

6.1.4.2 Chronic Kidney Disease

Diagnostic Challenges

- In chronic renal failure, half-life of GH is prolonged due to reduced metabolic clearance rate, which results in elevated GH levels depending on the degree of renal failure.
- Conversely, patients with ESRD on haemodialysis have decreased GH level leading to normal GH levels even in acromegaly patients.
- Oral glucose tolerance test (OGTT) can have paradoxical rise in GH levels in chronic renal failure.
- Moreover, uraemia leads to a state of GH resistance resulting in low IGF-1 generation. So, random GH, IGF-1 and OGTT can be misleading in patients with chronic renal failure with features of acromegaly.

Diagnosis

- A high serum IGF-1 level (>ULN) and high GH with features of acromegaly.
- Normal IGF-1 or GH levels with features of acromegaly in a patient with chronic renal failure do not rule out the diagnosis.

Tumour Localisation

MRI sella without contrast is the preferred modality.